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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶:

C12N 15/13, C07K 19/00, A61K 47/48,

C07K 16/24, C12N 15/85, 5/10

(11) International Publication Number:

WO 98/37200

(43) International Publication Date:

27 August 1998 (27.08.98)

(21) International Application Number:

PCT/US98/03337

(22) International Filing Date:

20 February 1998 (20.02.98)

(30) Priority Data:

08/804,444 09/012,116 21 February 1997 (21.02.97) US

22 January 1998 (22.01.98)

US US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application

US Filed on 09/012,116 (CIP) 22 January 1998 (22.01.98)

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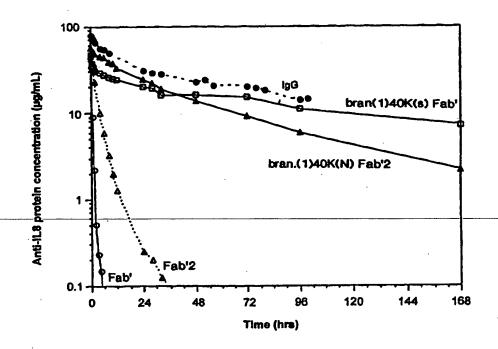
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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: ANTIBODY FRAGMENT-POLYMER CONJUGATES AND HUMANIZED ANTI-IL-8 MONOCLONAL ANTIBODIES



(57) Abstract

Humanized anti-IL-8 monoclonal antibodies and variants thereof are described for use in diagnostic applications and in the treatment of inflammatory disorders. Also described is a conjugate formed by an antibody fragment covalently attached to a non-proteinaceous polymer, wherein the apparent size of the conjugate is at least about 500 kD. The conjugate exhibits substantially improved half-life, mean residence time, and/or clearance rate in circulation as compared to the underivatized parental antibody fragment.

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ANTIBODY FRAGMENT-POLYMER CONJUGATES AND HUMANIZED ANTI-IL-8 MONOCLONAL ANTIBODIES

FIELD OF THE INVENTION

This application relates to the field of antibody fragments derivatized with polymers, and in particular to the use of such derivatization to increase the circulation half-lives of antibody fragment-polymer conjugates. This application also relates to humanized anti-interleukin-8 (IL-8) antibodies and to high affinity variants of such antibodies.

BACKGROUND

Modification of proteins with polyethylene glycol ("PEGylation") has the potential to increase residence time and reduce immunogenicity in vivo. For example, Knauf et al., J. Biol. Chem., 263: 15064-15070 (1988) reported a study of the pharmacodynamic behavior in rats of various polyoxylated glycerol and polyethylene glycol modified species of interleukin-2. Despite the known advantage of PEGylation, PEGylated proteins have not been widely exploited for clinical applications. In the case of antibody fragments, PEGylation has not been shown to extend serum half-life to useful levels. Delgado et al., Br. J. Cancer, 73: 175-182 (1996), Kitamura et al., Cancer Res., 51: 4310-4315 (1991), Kitamura et al., Biochem. Biophys. Res. Comm., 171: 1387-1394 (1990), and Pedley et al., Br. J. Cancer, 70: 1126-1130 (1994) reported studies characterizing blood clearance and tissue uptake of certain anti-tumor antigen antibodies or antibody fragments derivatized with low molecular weight (5 kD) PEG. Zapata et al., FASEB J., 9: A1479 (1995) reported that low molecular weight (5 or 10 kD) PEG attached to a sulfhydryl group in the hinge region of a Fab' fragment reduced clearance compared to the parental Fab' molecule.

Interleukin-8 (IL-8) is neutrophil chemotactic peptide secreted by a variety of cells in response to inflammatory mediators (for a review see Hebert et al. <u>Cancer Investigation</u> 11(6):743 (1993)). IL-8 can play an important role in the pathogenesis of inflammatory disorders, such as adult respiratory distress syndrome (ARDS), septic shock, and multiple organ failure. Immune therapy for such inflammatory disorders can include treatment of an affected patient with anti-IL-8 antibodies.

Sticherling et al. (J. Immunol. 143:1628 (1989)) disclose the production and characterization of four monoclonal antibodies against IL-8. WO 92/04372, published March 19, 1992, discloses polyclonal antibodies which react with the receptor-interacting site of IL-8 and peptide analogs of IL-8, along with the use of such antibodies to prevent an inflammatory response in patients. St. John et al. (Chest 103:932 (1993)) review immune therapy for ARDS, septic shock, and multiple organ failure, including the potential therapeutic-use-of-anti-IL-8-antibodies.—Sekido-et-al. (Nature 365:654 (1993)) disclose the prevention of lung reperfusion injury in rabbits by a monoclonal antibody against IL-8. Mulligan et al. (J. Immunol. 150:5585 (1993)), disclose protective effects of a murine monoclonal antibody to human IL-8 in inflammatory lung injury in rats.

WO 95/23865 (International Application No. PCT/US95/02589 published September 8, 1995) demonstrates that anti-IL-8 monoclonal antibodies can be used therapeutically in the treatment of other inflammatory disorders, such as bacterial pneumonias and inflammatory bowel disease.

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Anti-IL-8 antibodies are additionally useful as reagents for assaying IL-8. For example, Sticherling et al. (Arch. Dermatol. Res. 284:82 (1992)), disclose the use of anti-IL-8 monoclonal antibodies as reagents in immunohistochemical studies. Ko et al. (J. Immunol. Methods 149:227 (1992)) disclose the use of anti-IL-8 monoclonal antibodies as reagents in an enzyme-linked immunoabsorbent assay (ELISA) for IL-8.

SUMMARY OF THE INVENTION

One aspect of the invention is a conjugate consisting essentially of one or more antibody fragments covalently attached to one or more polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD.

Another aspect of the invention is an anti-IL-8 monoclonal antibody or antibody fragment comprising the complementarity determining regions of the 6G4.2.5LV11N35E light chain polypeptide amino acid sequence of Fig. 45 (SEQ ID NO:).

Further aspects of the invention are a nucleic acid molecule comprising a nucleic acid sequence encoding the above-described anti-IL-8 monoclonal antibody or antibody fragment; an expression vector comprising the nucleic acid molecule operably linked to control sequences recognized by a host cell transfected with the vector; a host cell transfected with the vector; and a method of producing the antibody fragment comprising culturing the host cell under conditions wherein the nucleic acid encoding the antibody fragment is expressed, thereby producing the antibody fragment, and recovering the antibody fragment from the host cell.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph depicting the blocking of IL-8 mediated elastase release from neutrophils by anti-IL-8 monoclonal antibody 5.12.14.

Figure 2 is a graph depicting the inhibition of ¹²⁵I-IL-8 binding to neutrophils by unlabeled IL-8.

Figure 3 demonstrates that a isotype matched negative control Fab (denoted as "4D5 Fab") does not inhibit the binding of ¹²⁵I-IL-8 to human neutrophils.

Figure 4 is a graph depicting the inhibition of binding of 125 I-IL-8 to human neutrophils by chimeric 5.12.14 Fab with an average IC₅₀ of 1.6 nM.

Figure 5 is a graph depicting the inhibition of binding of 125 I-IL-8 to human neutrophils by chimeric 6G.4.25 Fab with an average IC₅₀ of 7.5 nM.

Figure 6 demonstrates the inhibition of human IL-8 mediated neutrophil chemotaxis by chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab.

Figure 7 demonstrates the relative abilities of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab to inhibit rabbit IL-8 mediated neutrophil chemotaxis.

Figure 8 depicts the stimulation of elastase release from human neutrophils by various concentrations of human and rabbit IL-8. The relative extent of elastase release was quantitated by measurement of absorbance at 405 nm. The data represent mean ± SEM of triplicate samples.

Figure 9 is a graph depicting the ability of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab to inhibit elastase release from human neutrophils stimulated by human IL-8. The results were normalized to reflect the percentage of elastase release elicited by 100 nM IL-8 alone. The data represent the mean \pm SEM of three separate experiments performed on different days with different blood donors. IC₅₀ values were calculated by four parameter fit.

Figure 10 is a graph depicting the relative abilities of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab to inhibit elastase release from human neutrophils stimulated by rabbit IL-8. The results were normalized to reflect the percentage of elastase release elicited by 100 nM IL-8 alone. The data represent the mean \pm SEM of three separate experiments performed on different days with different blood donors. IC₅₀ values were calculated by four parameter fit.

Figures 11A-11J are a set of graphs depicting the following parameters in a rabbit ulcerative colitis model: Figure 11A depicts myeloperoxidase levels in tissue; Figure 11B depicts IL-8 levels in tissue; Figure 11C depicts colon weight; Figure 11D depicts gross inflammation; Figure 11E depicts edema; Figure 11F depicts extent of necrosis; Figure 11G depicts severity of necrosis; Figure 11H depicts neutrophil margination; Figure 11I depicts neutrophil infiltration; and Figure 11J depicts mononuclear infiltration.

Figure 12 is a graph depicting the effect of anti-IL-8 monoclonal antibody treatment on the number of neutrophils in bronchoalveolar lavage (BAL) fluid in animals infected with Streptococcus pneumoniae, Escherichia coli, or Pseudomonas aeruginosa. Treatment with 6G4.2.5 significantly reduced the number of neutrophils present in the BAL fluid compared to animals treated with isotype control mouse IgG (Figure 12).

Figure 13 depicts the DNA sequences (SEQ ID NOS: 1-6) of three primers designed for each of the light and heavy chains. Multiple primers were designed in order to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis for cloning the variable light and heavy regions of monoclonal antibody 5.12.14.

Figure 14 depicts the DNA sequences (SEQ ID NOS: 7-10) of one forward primer and one reverse primer for the 5.12.14 light chain variable region amplification.

Figure 15 depicts the DNA sequences (SEQ ID NOS: 11-18) of one forward primer and one reverse primer for the 5.12.14 heavy chain variable region amplification.

Figure 16 depicts the DNA sequence (SEQ ID NO: 19) and the amino acid sequence (SEQ ID NO: 20) of the 5.12.14 light chain variable region and partial murine constant light region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Important restriction sites are indicated in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 109. The partial murine constant light region is amino acids 110 to 123 (in italics).

Figure 17 depicts the DNA sequence (SEQ ID NO: 21) and the amino acid sequence (SEQ ID NO: 22) of the 5.12.14 heavy chain variable region and partial murine constant heavy region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison

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(amino acids denoted with asterisk). Important restriction sites are indicated in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 120. The partial murine constant heavy region is amino acids 121 to 130.

Figure 18 depicts the DNA sequences (SEQ ID NOS: 23-26) of amplification primers used to convert murine light and heavy chain constant region residues to their human equivalents.

Figure 19 depicts the DNA sequence (SEQ ID NO: 27) and the amino acid sequence (SEQ ID NO: 28) for the 5.12.14 light chain variable region and the human IgG1 light chain constant region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 109. The human constant light region is amino acids 110 to 215.

Figures 20A-20B depict the DNA sequence (SEQ ID NO: 29) and the amino acid sequence (SEQ ID NO: 30) for the 5.12.14 heavy chain variable region and the heavy chain constant region of human IgG1. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 120. The human constant heavy region is amino acids 121 to 229.

Figure 21 depicts the DNA sequences (SEQ ID NOS: 31-36) of three primers designed for each of the light and heavy chains. Multiple primers were designed in order to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis for cloning the variable light and heavy regions of monoclonal antibody 6G4.2.5.

Figure 22 depicts the DNA sequences (SEQ ID NOS: 37-40) of one forward primer and one reverse primer for the 6G4.2.5 light chain variable region amplification.

Figure 23 depicts the DNA sequences (SEQ ID NOS: 41-46) of one forward primer and one reverse primer for the 6G4.2.5 heavy chain variable region amplification.

Figure 24 depicts the DNA sequence (SEQ ID NO: 47) and the amino acid sequence (SEQ ID NO: 48) of the 6G4.2.5 light chain variable region and partial murine constant light region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Useful cloning sites are in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 114. The partial murine constant light region is amino acids 115 to 131.

Figure 25 depicts the DNA sequence (SEQ ID NO: 49) and the amino acid sequence (SEQ ID NO: 50) of the 6G4.2.5 heavy chain variable region and partial murine constant heavy region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Useful cloning sites are in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 122. The partial murine constant heavy region is amino acids 123 to 135.

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Figure 26 depicts the DNA sequences (SEQ ID NOS: 51-54) of primers to convert the murine light chain and heavy chain constant regions to their human equivalents.

Figures 27A-27B depict the DNA sequence (SEQ ID NO: 55) and the amino acid sequence (SEQ ID NO: 56) for the chimeric 6G4.2.5 light chain. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 114. The human constant heavy region is amino acids 115 to 220.

Figures 28A-28B depict the DNA sequence (SEQ ID NO: 57) and the amino acid sequence (SEQ ID NO: 58) for the chimeric 6G4.2.5 heavy chain. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 122. The human constant heavy region is amino acids 123 to 231.

Fig. 29 depicts an amino acid sequence alignment of murine 6G425 light chain variable domain (SEQ ID NO: 59), humanized 6G425 F(ab)-1 light chain variable domain (SEQ ID NO: 60), and human light chain kI consensus framework (SEQ ID NO: 61) amino acid sequences, and an amino acid sequence alignment of murine 6G425 heavy chain variable domain (SEQ ID NO: 62), humanized 6G425 F(ab)-1 heavy chain variable domain (SEQ ID NO: 63), and human IgG1 subgroup III heavy chain variable domain (SEQ ID NO: 64) amino acid sequences, used in the humanization of 6G425. Light chain CDRs are labeled L1, L2, L3; heavy chain CDRs are labeled H1, H2, and H3. = and + indicate CDR sequences as defined by X-ray crystallographic contacts and sequence hypervariability, respectively. # indicates a difference between the aligned sequences. Residue numbering is according to Kabat *et al.* Lower case lettering denotes the insertion of an amino acid residue relative to the humIII consensus sequence numbering.

Fig. 30 is a graph with three panels (A, B and C) depicting the ability of F(ab)-9 (humanized 6G4V11 Fab) to inhibit human wild type IL-8, human monomeric IL-8, and rhesus IL-8 mediated neutrophil chemotaxis, respectively. Panel A presents inhibition data for F(ab)-9 samples at concentrations of 0.06 nM, 6.25 nM, 12.5 nM, 25 nM, 50 nM, and 100 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 100 nM, and for a no antibody control sample, in the presence of 2nM human wild type IL-8. Panel B presents inhibition data for F(ab)-9 samples at concentrations of 6.25 nM, 12.5 nM, 25 nM, and 50 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 100 nM, and for a no antibody control sample, in the presence of 4 nM human monomeric IL-8 (denoted as "BD59" and as "monomeric IL-8"). Panel C presents inhibition data for F(ab)-9 samples at concentrations of 1 nM, 12.5 nM, 25 nM, and 50 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 100 nM, and for a no antibody control sample, in the presence of 2 nM rhesus IL-8. In addition, all panels A, B an C each presents data for a no IL-8 buffer control sample (denoted as "Buffer") in the respective inhibition assay.

Fig. 31A depicts the amino acid sequences of the humanized anti-IL-8 6G4.2.5V11 light chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 65), the humanized anti-IL-8 6G4.2.5V11

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heavy chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 66), and a peptide linker in a C-terminal fusion with M13 phage gene-III coat protein (SEQ ID NO: 67).

Fig. 31B depicts the nucleic acid sequence (SEQ ID NO: 68) and the translated amino acid sequence (SEQ ID NO: 65) of the humanized anti-IL-8 6G4.2.5V11 light chain in an N-terminal fusion with the STII leader peptide.

Fig. 31C depicts the amino acid sequences of the humanized anti-IL-8 6G4.2.5V19 light chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 69), and the humanized anti-IL-8 6G4.2.5V19 heavy chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 70).

Fig. 32 is a three dimensional computer model of the humanized anti-IL-8 6G4.2.5V11 antibody. Heavy chain CDR loops and variable domain regions appear in purple, and CDR-H3 side chain residues appear in yellow. Heavy chain constant domain regions appear in red. Light chain CDR loops and variable domain regions appear in off-white, and the Asn residue at amino acid position 35 (N35) in CDR L1 appears in green. Light chain constant domain regions appear in amber.

Fig. 33 is a Scatchard plot depicting the inhibition of ¹²⁵I-IL-8 binding to human neutrophils exhibited by intact murine 6G4.2.5 antibody (denoted 6G4 murine mAb), 6G4.2.5 murine-human chimera Fab (denoted 6G4 chimera), humanized 6G4.2.5 Fab versions 1 and 11 (denoted V1 and V11), and variant 6G4.2.5V11N35A Fab (denoted V11N35A).

Fig. 34 is a graph with four panels (A, B, C, and D) depicting the ability of 6G4.2.5V11N35A Fab to inhibit human wild type IL-8, human monomeric IL-8, rabbit IL-8, and rhesus IL-8 mediated neutrophil chemotaxis, respectively. Panel A presents inhibition data for 6G4.2.5V11N35A Fab samples at concentrations of 0.5, 1, 2, 4, 8, 16, and 33 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 33 nM, and for a no antibody control (denoted "HuIL-8") sample, in the presence of 2 nM human wild type IL-8. Panel B presents inhibition data for 6G4.2.5V11N35A Fab samples at concentrations of 0.5, 1, 2, 4, 8, 16, and 33 nM, for an intact 6G4.2.5 mAb sample at a concentration of 33 nM, for an isotype control antibody (denoted as "4D5") sample at a concentration of 33 nM, and for a no antibody control (denoted "BD59") sample, in the presence of 2 nM human monomeric IL-8. Panel C presents inhibition data for 6G4.2.5V11N35A Fab samples at concentrations of 0.5, 1, 2, 4, 8, 16, and 33 nM, for an intact 6G4.2.5 mAb sample at a concentration of 33 nM, for an isotype control antibody (denoted "4D5") sample at a concentration of 33 nM, and for a no antibody control (denoted "Rab IL-8") sample, in the presence of 2 nM rabbit IL-8. Panel D presents inhibition data for 6G4.2.5V11N35A Fab samples at concentrations of 0.5, 1, 2, 4, 8, 16, and 33 nM, for an intact 6G4.2.5 mAb sample at a concentration of 33 nM, for an isotype control antibody (denoted as "4D5") sample at a concentration of 33 nM, and for a no antibody control (denoted "Rhe IL-8") sample, in the presence of 2 nM rhesus IL-8. In addition, panels B, C and D each presents data for human wild type IL-8 control (denoted "HuIL-8") samples at a concentration of 2 nM in the respective assay, and panels A, B, C, and D each presents data for a no IL-8 buffer control (denoted "Buffer") sample in the respective assay.

Fig. 35 depicts the amino acid sequences of the humanized anti-IL-8 6G4.2.5V11N35A light chain

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in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 71), the humanized anti-IL-8 6G4.2.5V11N35A heavy chain in an N-terminal fusion with the STII leader peptide (SEQ ID NO: 66), and the GCN4 leucine zipper peptide (SEQ ID NO: 72). The Ala residue (substituted for the wild type Asn residue) at amino acid position 35 in the 6G4.2.5V11N35A light chain appears in bold case. A putative pepsin cleavage site in the GCN4 leucine zipper sequence is underlined.

Fig. 36 depicts the DNA sequence (SEQ ID NO: 73) and the amino acid sequence (SEQ ID NO: 71) of the humanized anti-IL-8 6G4.2.5V11N35A light chain in an N-terminal fusion with the STII leader peptide. Complementarity determining regions L1, L2, and L3 are underlined

Figs. 37A-37B depict the DNA sequence (SEQ ID NO: 74) and the amino acid sequence (SEQ ID NO: 75) of the humanized anti-IL-8 6G4.2.5V11N35A heavy chain in an N-terminal fusion with the STII leader peptide and in a C-terminal fusion with the GCN4 leucine zipper sequence. Complementarity determining regions H1, H2, and H3 are underlined.

Fig. 38 is a Scatchard plot depicting the inhibition of ¹²⁵I-IL-8 binding to human neutrophils exhibited by 6G4.2.5V11N35A Fab (denoted Fab), 6G4.2.5V11N35A F(ab')₂ (denoted F(ab')₂), and human wild type IL-8 control (denoted IL-8).

Fig. 39 is a graph depicting a comparison of the wild type human IL-8 mediated neutrophil chemotaxis inhibition activities of the 6G4.2.5V11N35A F(ab')₂ and 6G4.2.5V11N35A Fab. Inhibition data are presented for 6G4.2.5V11N35A Fab samples (denoted "N35A Fab") and 6G4.2.5V11N35A F(ab')₂ samples (denoted N35A F(ab')₂) at concentrations of 0.3, 1, 3, 10, 30, and 100 nM, for an isotype control antibody (denoted as "4D5") sample at a concentration of 100 nM, and for a no antibody control sample, in the presence of 2 nM human wild type IL-8. In addition, inhibition data are presented for no IL-8 buffer control samples (denoted "Buffer").

Fig. 40 is a graph depicting the ability of 6G4.2.5V11N35A F(ab')₂ to inhibit human monomeric IL-8, rhesus IL-8, and rabbit IL-8 mediated neutrophil chemotaxis. Human monomeric IL-8 mediated neutrophil chemotaxis data are presented for 6G4.2.5V11N35A F(ab')₂ samples at concentrations of 0.3, 1, 3, and 10 nM, for an isotype control antibody (denoted as "4D5") sample at a concentration of 100 nM, and for a no antibody control sample (denoted as "BD59"), in the presence of human monomeric IL-8 (denoted as "BD59") at a concentration of 0.5 nM. Rhesus IL-8 mediated neutrophil chemotaxis data are presented for 6G4.2.5V11N35A F(ab')₂ samples at concentrations of 0.3, 1, 3, and 10 nM, and for a no antibody control sample, in the presence of rhesus IL-8 at a concentration of 2 nM. Rabbit IL-8 mediated neutrophil chemotaxis data are presented for 6G4.2.5V11N35A F(ab')₂ samples at concentrations of 0.3, 1, 3, and 10 nM, and for a no antibody control sample, in the presence of rabbit IL-8 at a concentration of 2 nM. In addition, inhibition data are presented for a no IL-8 buffer control sample (denoted as "Buffer") and for a 2 nM human wild type IL-8 (denoted as "HuIL-8").

Figs. 41A-41Q depict the nucleic acid sequence (SEQ ID NO: 76) of the p6G4V11N35A.F(ab')2

vector.

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Fig. 42 depicts the nucleic acid sequences of the stop template primer (SEQ ID NO:) and the NNS randomization primer (SEQ ID NO:) used for random mutagenesis of amino acid position 35 in variable light chain CDR-L1 of humanized antibody 6G4V11.

Fig. 43A is a table of data describing the frequencies of different phage display clones obtained from the randomization of amino acid position 35 in variable light chain CDR-L1 of humanized antibody 6G4V11.

Fig. 43B contains graphs of displacement curves depicting the inhibition of 125I-IL-8 binding to neutrophils exhibited by the 6G4V11N35A, 6G4V11N35D, 6G4V11N35E and 6G4V11N35G Fab's.

Fig. 44 contains a graph depicting the typical kinetics of an anti-IL-8 antibody fragment (6G4V11N35A F(ab')2) binding to IL-8. Fig. 44 also contains a table of data providing the equilibrium constant for 6G4V11N35A Fab binding to 1L-8 (rate constants were not determined "ND"), and the equilibrium and rate constants for 6G4V11N35A F(ab')2 and 6G4V11N35E Fab binding to IL-8.

Fig. 45 depicts the DNA sequence (SEQ ID NO:) and amino acid sequence (SEQ ID NO:)of the 6G4V11N35E light chain in an N-terminal fusion with the STII leader peptide. Complementarity determining regions L1, L2 and L3 are underlined.

Fig. 46 is a graph depicting the ability of 6G4V11N35E Fab to inhibit human IL-8 (dark columns) and rabbit IL-8 (light columns) mediated neutrophil chemotaxis. Data are presented for 6G4V11N35E Fab samples at concentrations of 0.4, 1.2, 3.7, 11 and 33 nM, and for an isotype control antibody (4D5) sample at a concentration of 100 nM, in the presence of 2 nM human IL-8 or 2 nM rabbit IL-8. In addition, inhibition data are presented for a no IL-8 buffer control sample (denoted "Buffer") and for human and rabbit IL-8 control samples (denoted "IL-8").

Fig. 47 depicts the DNA sequence of the sense (SEQ ID NO:) and anti-sense (SEQ ID NO:) strands of a PvuII-Xhol synthetic nucleotide encoding amino acids Leu4 to Phe29 of the 6G4V11N35A heavy chain.

Figs. 48A-48T depict the DNA sequence (SEQ ID NO:) of plasmid p6G4V11N35A.choSD9.

Fig. 49 contains graphs of displacement curves depicting the inhibition of ¹²⁵I-IL-8 binding to neutrophils exhibited by the full length IgG1 forms of variants 6G4V11N35A and 6G4V11N35E.

Figs. 50A-50B are graphs depicting the ability of full length 6G4V11N35A IgG1 and 6G4V11N35E lgG1 to inhibit human IL-8 (Fig. 50A) and rabbit IL-8 (Fig. 50B) mediated neutrophil chemotaxis.

Fig. 51 contains a graph depicting the typical kinetics of a full length anti-IL8 antibody (6G4V11N35A lgG1) binding to IL-8. Fig. 51 also contains a table of data providing the equilibrium and rate constants for full length murine 6G4.2.5 IgG2a, 6G4V11N35A IgG1 and 6G4V11N35E IgG1 binding to IL-8.

Fig. 52 contains graphs of displacement curves depicting the results of an unlabeled IL-8/¹²⁵l-IL-8 competition radioimmunoassay performed with full-length-6G4V11N35A-lgG1-and-6G4V11N35E-lgG1.

Fig. 53 depicts the DNA sequence (SEQ ID NO:) and amino acid sequence (SEQ ID NO:) of the 6G4V11N35A Fab' heavy chain (6G4V11N35A Fab heavy chain modified to contain a cysteine residue in the hinge region).

Figs. 54A-54C contain graphs of displacement curves depicting the IL-8 binding and IC₅₀'s for PEG-maleimide modified 6G4V11N35A Fab' molecules.

Figs. 55A-55C are graphs depicting the ability of PEG-maleimide modified 6G4V11N35A Fab' molecules to inhibit human IL-8 and rabbit IL-8 mediated neutrophil chemotaxis.

Figs. 56A-56C are graphs depicting the ability of PEG-maleimide modified 6G4V11N35A Fab' molecules to inhibit IL-8 mediated release of β-glucuronidase from neutrophils.

Figs. 57A-57B contain graphs of displacement curves depicting the inhibition of ¹²⁵I-IL-8 binding to neutrophils exhibited by PEG-succinimide modified 6G4V11N35A Fab'₂ molecules.

Figs. 58A-58B are graphs depicting the ability of PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules to inhibit human IL-8 mediated neutrophil chemotaxis.

Figs. 59A-59B are graphs depicting the ability of PEG-succinimide modified 6G4V11N35A $F(ab')_2$ molecules to inhibit human IL-8 mediated release of β -glucuronidase from neutrophils.

Fig. 60 is a graph depicting the theoretical molecular weight (dotted bars) and effective size (solid bars) of PEG-maleimide modified 6G4V11N35A Fab' molecules as determined by SEC-HPLC.

Fig. 61 is an SDS-PAGE gel depicting the electrophoretic mobility of various PEG-maleimide modified 6G4V11N35A Fab' molecules.

Fig. 62 contains size exclusion chromatograms (SEC-HPLC) depicting the retention times and effective (hydrodynamic) sizes of various PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules.

Fig. 63 is a graph depicting the theoretical molecular weight (open columns), effective size determined by SEC-HPLC (solid columns), and the actual molecular weight determined by SEC-light scattering (shaded columns) for various PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules.

Fig. 64 is an SDS-PAGE gel depicting the electrophoretic mobility of various PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules. From left to right, lane 1 contains unmodified F(ab')₂, lane 2 contains F(ab')₂ coupled to two 40 kD branched PEG-succinimide molecules (denoted "Br(2)-40kD(N)-F(ab')2"), lane 3 contains F(ab')₂ coupled to one 40 kD branched PEG-succinimide molecule (denoted "Br(1)-40kD-(N)-Fab'2"), lane 4 contains a mixture of F(ab')₂ coupled to four 20 kD linear PEG-succinimide molecules and F(ab')₂ coupled to five 20 kD linear PEG-succinimide molecules (denoted

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"L(4+5)-20kD-(N)-Fab'2"), lane 5 contains F(ab')₂ coupled to one 20 kD linear PEG-succinimide molecule (denoted "L(1)-20kD-(N)-Fab'2"), and lane 6 contains molecular weight standards.

Fig. 65 contains graphs comparing the serum concentration vs. time profiles of various PEG-maleimide modified 6G4V11N35A Fab' molecules (upper graph) and various PEG-succinimide modified 6G4V11N35A F(ab')₂ molecules (lower graph) in rabbits. In the upper graph, "bran.(1)40K(s)Fab' " denotes 6G4V11N35A Fab' coupled to one 40 kD branched PEG-maleimide molecule, "lin.(1)40K(s)Fab' " denotes 6G4V11N35A Fab' coupled to one 40 kD linear PEG-maleimide molecule, "lin.(1)30K(s)Fab' " denotes 6G4V11N35A Fab' coupled to one 30 kD linear PEG-maleimide molecule, "lin.(1)20K(s)Fab'' denotes 6G4V11N35A Fab' coupled to one 20 kD linear PEG-maleimide molecule. In the lower graph, "bran.(2)40K(N)Fab'2" denotes 6G4V11N35A F(ab')₂ coupled to two 40 kD branched PEG-succinimide molecules, "bran.(1)40K(N)Fab'2" denotes 6G4V11N35A F(ab')₂ coupled to one 40 kD branched PEG-succinimide molecules, "bran.(1)40K(N)Fab'2" denotes unmodified 6G4V11N35A F(ab')₂. In both graphs, "lgG" denotes a full length lgG1 equivalent of the human-murine chimeric anti-rabbit IL-8 Fab described in Example F below.

Fig. 66 contains graphs comparing the serum concentration vs. time profiles of 6G4V11N35A Fab' coupled to one 40 kD branched PEG-maleimide molecule (denoted as "bran.(1)40K(s)Fab'"), 6G4V11N35A F(ab')₂ coupled to one 40 kD branched PEG-succinimide molecule (denoted as "bran.(1)40K(N)Fab'2"), unmodified 6G4V11N35A F(ab')₂ (denoted as "Fab'2"), unmodified 6G4V11N35A Fab' (denoted as "Fab'"), and a full length IgG1 (denoted as "IgG") equivalent of the human-murine chimeric anti-rabbit IL-8 Fab described in Example F below.

Fig. 67 is a graph depicting the effect of 6G4V11N35A Fab' coupled to one 40 kD branched PEG-maleimide molecule (denoted as "PEG 40 Kd") and murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5 (full length IgG2a) (denoted as "6G4.2.5") on gross weight of entire lung in an ARDS rabbit model.

Fig. 68 is a graph depicting the effect of 6G4V11N35A Fab' coupled to one branched 40 kD PEG-maleimide molecule (denoted as "PEG 40 Kd") and murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5 (full length IgG2a) (denoted as "6G4.2.5") on BAL total leukocyte (light columns) and polymorphonuclear cell (dark columns) counts in an ARDS rabbit model. Untreated (no therapeutics) control animal data is denoted as "Control".

Fig. 69 is a graph depicting the effect of 6G4V11N35A Fab' coupled to one branched 40 kD PEG-maleimide molecule (denoted as "PEG 40 Kd") and murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5 (full length lgG2a) (denoted as "6G4.2.5") on PaO2/FiO2 ratio at 24 hours-post treatment (light columns) and 48 hours post-treatment (dark columns) in an ARDS rabbit model. Untreated (no therapeutics) control animal data is denoted as "Control".

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DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. DEFINITIONS

In general, the following words or phrases have the indicated definition when used in the description, examples, and claims.

"Polymerase chain reaction" or "PCR" refers to a procedure or technique in which minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Patent No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers can coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis *et al.*, Cold Spring Harbor Symp. Quant. Biol. 51:263 (1987); Erlich, ed., PCR Technology (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample comprising the use of a known nucleic acid as a primer and a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

"Native antibodies and immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Clothia et al., J. Mol. Biol. 186:651 (1985); Novotny and Haber, Proc. Natl. Acad. Sci. U.S.A. 82:4592 (1985)).

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly

conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species (scFv), one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. For a review of scFv see Pluckthun, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (k) and lambda (l), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy-chain constant domains that correspond to the different

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classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies) and antibody compositions with polyepitopic specificity.

"Antibody fragment", and all grammatical variants thereof, as used herein are defined as a portion of an intact antibody comprising the antigen binding site or variable region of the intact antibody, wherein the portion is free of the constant heavy chain domains (i.e. CH2, CH3, and CH4, depending on antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'-SH, F(ab')2, and Fv fragments; diabodies; any antibody fragment that is a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a "single-chain antibody fragment" or "single chain polypeptide"), including without limitation (1)single-chain Fv (scFv) molecules (2)single chain polypeptides containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety and (3)single chain polypeptides containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light chain moiety; and multispecific or multivalent structures formed from antibody fragments. In an antibody fragment comprising one or more heavy chains, the heavy chain(s) can contain any constant domain sequence (e.g. CH1 in the IgG isotype) found in a non-Fc region of an intact antibody, and/or can contain any hinge region sequence found in an intact antibody, and/or can contain a leucine zipper sequence fused to or situated in the hinge region sequence or the constant domain sequence of the heavy chain(s). Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney et al., J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below.

Unless specifically indicated to the contrary, the term "conjugate" as described and claimed herein is defined as a heterogeneous molecule formed by the covalent attachment of one or more antibody fragment(s) to one or more polymer molecule(s), wherein the heterogeneous molecule is water soluble, i.e. soluble in physiological fluids such as blood, and wherein the heterogeneous molecule is free of any structured aggregate. In the context of the foregoing definition, the term "structured aggregate" refers to (1) any aggregate of molecules in aqueous solution having a spheroid or spheroid shell structure, such that the heterogeneous molecule is not in a micelle or other emulsion structure, and is not anchored to a lipid bilayer, vesicle or liposome; and (2) any aggregate of molecules in solid or insolubilized form, such as a chromatography bead matrix, that does not release the heterogeneous molecule into solution upon contact with an aqueous phase. Accordingly, the term "conjugate" as defined herein encompasses the aforementioned heterogeneous molecule in a precipitate, sediment, bioerodible matrix or other solid capable of releasing the heterogeneous molecule into aqueous solution upon hydration of the solid.

Unless specifically indicated to the contrary, the terms "polymer", "polymer molecule", "nonproteinaceous polymer", and "nonproteinaceous polymer molecule" are used interchangeably and are

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defined as a molecule formed by covalent linkage of two or more monomers, wherein none of the monomers is contained in the group consisting of alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), leucine (Leu), methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), arginine (Arg), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), and tyrosine (Tyr) residues.

The term "monoclonal antibody" (mAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they can be synthesized by hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567 to Cabilly et al.). The "monoclonal antibodies" also include clones of antigen-recognition and binding-site containing antibody fragments (Fv clones) isolated from phage antibody libraries using the techniques described in Clackson et al., Nature, 352:624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597 (1991), for example.

The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-IL-8 antibody with a constant domain (e.g. "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, F(ab')₂, and Fv), so long as they exhibit the desired biological activity. (See, e.g., U.S. Pat. No. 4,816,567 to Cabilly *et al.*; Mage and Lamoyi, in Monoclonal Antibody Production Techniques and Applications, pp. 79-97 (Marcel Dekker, Inc., New York, 1987).)

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly *et al.*, supra; Morrison *et al.*, Proc. Natl. Acad. Sci. U.S.A. 81:6851 (1984)).

"Humanized" forms of non-human (e.g., murine) antibodies are specific chimeric

immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂, or other antigen-binding subsequences-of-antibodies)-which-contain-minimal sequence-derived-from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones et al., Nature 332:323 (1988); and Presta, Curr. Op. Struct. Biol. 2:593 (1992).

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"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal herein is human.

As used herein, protein, peptide and polypeptide are used interchangeably to denote an amino acid polymer or a set of two or more interacting or bound amino acid polymers.

As used herein, the term "inflammatory disorders" refers to pathological states resulting in inflammation, typically caused by neutrophil chemotaxis. Examples of such disorders include inflammatory skin diseases including psoriasis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); ischemic reperfusion; adult respiratory distress syndrome; dermatitis; meningitis; encephalitis; uveitis; autoimmune diseases such as rheumatoid arthritis, Sjorgen's syndrome, vasculitis; diseases involving leukocyte diapedesis; central nervous system (CNS) inflammatory disorder, multiple organ injury syndrome secondary to septicaemia or trauma; alcoholic hepatitis, bacterial pneumonia, antigen-antibody complex mediated diseases; inflammations of the lung, including pleurisy, alveolitis, vasculitis, pneumonia, chronic bronchitis, bronchiectasis, and cystic fibrosis; etc. The preferred indications are bacterial pneumonia and inflammatory bowel disease such as ulcerative colitis.

The terms "hydrodynamic size", "apparent size", "apparent molecular weight", "effective size" and "effective molecular weight" of a molecule are used synonymously herein refer to the size of a molecule as determined by comparison to a standard curve produced with globular protein molecular weight standards in a size exclusion chromatography system, wherein the standard curve is created by mapping the actual

molecular weight of each standard against its elution time observed in the size exclusion chromatography system. Thus, the apparent size of a test molecule is derived by using the molecule's elution time to extrapolate a putative molecular weight from the standard curve. Preferably, the molecular weight standards used to create the standard curve are selected such that the apparent size of the test molecule falls within the linear portion of the standard curve.

II. MODES FOR CARRYING OUT THE INVENTION

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In one part, the invention arises from the surprising and unexpected discovery that antibody fragment-polymer conjugates having an effective or apparent size significantly greater than the antibody fragment-polymer conjugates described in the art confers an increase in serum half-life, an increase in mean residence time in circulation (MRT), and/or a decrease in serum clearance rate over underivatized antibody fragment which far exceed the modest changes in such biological property or properties obtained with the art-known antibody fragment-polymer conjugates. The present inventors have determined for the first time that increasing the effective size of an antibody fragment to at least about 500,000 D, or increasing the effective size of an antibody fragment by at least about 8 fold over the effective size of the parental antibody fragment, or derivatizing an antibody fragment with a polymer of at least about 20,000 D in molecular weight, yields a molecule with a commercially useful pharmacokinetic profile. The greatly extended serum half-life, extended MRT, and/or reduced serum clearance rate of the conjugates of the invention makes such conjugates viable alternatives to intact antibodies used for therapeutic treatment of many disease indications. Antibody fragments provide significant advantages over intact antibodies, notably the fact that recombinant antibody fragments can be made in bacterial cell expression systems. Bacterial cell expression systems provide several advantages over mammalian cell expression systems, including reduced time and cost at both the research and development and manufacturing stages of a product.

In another part, the present invention also arises from the humanization of the 6G4.2.5 murine antirabbit IL-8 monoclonal antibody ("6G4.2.5") described in WO 95/23865 (PCT/US95/02589 published September 8, 1995), the entire disclosure of which is specifically incorporated herein by reference. The hybridoma producing antibody 6G4.2.5 was deposited on September 28, 1994 with the American Type Culture Collection and assigned ATCC Accession No. HB 11722 as described in the Examples below. In one aspect, the invention provides a humanized derivative of the 6G4.2.5 antibody, variant 11 (referred to herein as "6G4.2.5v11"), in which the murine CDRs of 6G4.2.5 are grafted onto a consensus framework for human light chain k1 and human IgG1 heavy chain subgroup III, followed by importing three framework residues from the murine 6G4.2.5 parent heavy chain variable domain sequence into analogous sites in the heavy chain variable domain of the human template sequence, as described in the Examples below. In another aspect, the invention provides variants of the 6G4.2.5v11 antibody with certain amino acid substitution(s) yielding increased affinity for human IL-8 and/or promoting greater efficiency in recombinant manufacturing processes.

It will be understood that in the context of this Section (II) and all subsections thereof, every reference to "an antibody fragment" or "the antibody fragment" contained in a conjugate shall be a reference

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to one or more antibody fragment(s) in the conjugate (consistent with the definition of the term "conjugate" set forth in Section (I) above), except where the number of antibody fragment(s) in the conjugate is expressly indicated. It will be understood that in the context of this Section (II) and all subsections thereof, every reference to "a polymer", "a polymer molecule", "the polymer", or "the polymer molecule" contained in a conjugate shall be a reference to one or more polymer molecule(s) in the conjugate (consistent with the definition of the term "conjugate" set forth in Section (I) above), except where the number of polymer molecule(s) in the conjugate is expressly indicated.

1. LARGE EFFECTIVE SIZE ANTIBODY FRAGMENT-POLYMER CONJUGATES

In one aspect, the invention provides an antibody fragment covalently attached to a polymer to form a conjugate having an effective or apparent size of at least about 500,000 Daltons (D). In another aspect, the invention provides an antibody fragment covalently attached to a polymer to form a conjugate having an apparent size that is at least about 8 fold greater than the apparent size of the parental antibody fragment. In yet another aspect, the invention provides an antibody fragment covalently attached to a polymer of at least about 20,000 D in molecular weight (MW). It will be appreciated that the unexpectedly and surprisingly large increase in antibody fragment serum half-life, increase in MRT, and/or decrease in serum clearance rate can be achieved by using any type of polymer or number of polymer molecules which will provide the conjugate with an effective size of at least about 500,000 D, or by using any type of polymer or number of polymer molecules which will provide the conjugate with an effective size that is at least about 8 fold greater than the effective size of the parental antibody fragment, or by using any type or number of polymers wherein each polymer molecule is at least about 20,000 D in MW. Thus, the invention is not dependent on the use of any particular polymer or molar ratio of polymer to antibody fragment in the conjugate.

In addition, the beneficial aspects of the invention extend to antibody fragments without regard to antigen specificity. Although variations from antibody to antibody are to be expected, the antigen specificity of a given antibody will not substantially impair the extraordinary improvement in serum half-life, MRT, and/or serum clearance rate for antibody fragments thereof that can be obtained by derivatizing the antibody fragments as taught herein.

In one embodiment, the conjugate has an effective size of at least about 500,000 D, or at least about 800,000 D, or at least about 1,000,000 D, or at least about 1,200,000 D, or at least about 1,400,000 D, or at least about 1,500,000 D, or at least about 1,800,000 D, or at least about 2,000,000 D, or at least about 2,500,000 D.

In another embodiment, the conjugate has an effective size of at or about 500,000 D to at or about 10,000,000 D, or an effective size of at or about 500,000 D to at or about 8,000,000 D, or an effective size of at or about 500,000 D to at or about 500,000 D to at or about 4,000,000 D, or an effective size of at or about 3,000,000 D, or an effective size of at or about 500,000 D to at or about 500,000 D, or an effective size of at or about 500,000 D, or an effective size of at or about 1,800,000 D, or an effective size of at or about 1,800,000 D, or an

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effective size of at or about 500,000 D to at or about 1,600,000 D, or an effective size of at or about 500,000 D to at or about 1,500,000 D, or an effective size of at or about 500,000 D to at or about 1,000,000 D.

In another embodiment, the conjugate has an effective size of at or about 800,000 D to at or about 10,000,000 D, or an effective size of at or about 800,000 D to at or about 8,000,000 D, or an effective size of at or about 800,000 D to at or about 5,000,000 D, or an effective size of at or about 3,000,000 D, or an effective size of at or about 3,000,000 D, or an effective size of at or about 800,000 D to at or about 800,000 D to at or about 800,000 D to at or about 2,500,000 D, or an effective size of at or about 1,800,000 D, or an effective size of at or about 1,800,000 D, or an effective size of at or about 800,000 D to at or about 1,500,000 D, or an effective size of at or about 1,500,000 D, or an effective size of at or about 1,000,000 D.

In another embodiment, the conjugate has an effective size of at or about 900,000 D to at or about 10,000,000 D, or an effective size of at or about 900,000 D to at or about 8,000,000 D, or an effective size of at or about 900,000 D to at or about 900,000 D to at or about 4,000,000 D, or an effective size of at or about 900,000 D to at or about 2,000,000 D, or an effective size of at or about 900,000 D to at or about 2,000,000 D, or an effective size of at or about 1,800,000 D, or an effective size of at or about 900,000 D to at or about 1,800,000 D, or an effective size of at or about 1,500,000 D.

In another embodiment, the conjugate has an effective size of at or about 1,000,000 D to at or about 10,000,000 D, or an effective size of at or about 1,000,000 D to at or about 8,000,000 D, or an effective size of at or about 1,000,000 D to at or about 5,000,000 D, or an effective size of at or about 1,000,000 D to at or about 1,000,000 D to at or about 3,000,000 D, or an effective size of at or about 1,000,000 D, or an effective size of at or about 2,500,000 D, or an effective size of at or about 1,000,000 D to at or about 1,000,000 D to at or about 1,000,000 D to at or about 1,000,000 D, or an effective size of at or about 1,000,000 D to at or about 1,000,000 D, or an effective size of at or about 1,000,000 D to at or about 1,000,000 D to at or about 1,500,000 D.

In a further embodiment, the conjugate has an effective size that is at least about 8 fold greater, or at least about 10 fold greater, or at least about 12 fold greater, or at least about 15 fold greater, or at least about 25 fold greater, or at least about 28 fold greater, or at least about 30 fold greater, or at least about 40 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 8 fold to about 100 fold greater, or is about 8 fold to about 80 fold greater, or is about 8 fold to about 50 fold greater, or is about 8 fold to about 40 fold greater, or is about 8 fold to about 30 fold greater, or is about 8 fold to about 28 fold greater, or is about 8 fold to about 25 fold greater, or is about 8 fold to about 20 fold greater, or is about 8 fold to about 18 fold greater, or is about 8 fold to about 18 fold greater, or is about 8 fold to about 15 fold greater, than the effective size of the parental antibody fragment.

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In another embodiment, the conjugate has an effective size that is about 12 fold to about 100 fold greater, or is about 12 fold to about 80 fold greater, or is about 12 fold to about 50 fold greater, or is about 12 fold to about 40 fold greater, or is about 12 fold to about 30 fold greater, or is about 12 fold to about 28 fold greater, or is about 12 fold to about 25 fold greater, or is about 12 fold to about 20 fold greater, or is about 12 fold to about 18 fold greater, or is about 15 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 15 fold to about 100 fold greater, or is about 15 fold to about 80 fold greater, or is about 15 fold to about 50 fold greater, or is about 15 fold to about 40 fold greater, or is about 15 fold to about 30 fold greater, or is about 15 fold to about 28 fold greater, or is about 15 fold to about 25 fold greater, or is about 15 fold to about 20 fold greater, or is about 15 fold to about 18 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 18 fold to about 100 fold greater, or is about 18 fold to about 80 fold greater, or is about 18 fold to about 50 fold greater, or is about 18 fold to about 40 fold greater, or is about 18 fold to about 30 fold greater, or is about 18 fold to about 28 fold greater, or is about 18 fold to about 25 fold greater, or is about 18 fold to about 20 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 20 fold to about 100 fold greater, or is about 20 fold to about 80 fold greater, or is about 20 fold to about 50 fold greater, or is about 20 fold to about 40 fold greater, or is about 20 fold to about 30 fold greater, or is about 20 fold to about 28 fold greater, or is about 20 fold to about 25 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 25 fold to about 100 fold greater, or is about 25 fold to about 25 fold to about 50 fold greater, or is about 25 fold to about 40 fold greater, or is about 25 fold to about 30 fold greater, or is about 25 fold to about 25 fold to about 25 fold to about 25 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 28 fold to about 100 fold greater, or is about 28 fold to about 80 fold greater, or is about 28 fold to about 50 fold greater, or is about 28 fold to about 40 fold greater, or is about 28 fold to about 30 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 30 fold to about 100 fold greater, or is about 30 fold to about 50 fold greater, or is about 30 fold to about 40 fold greater, than the effective size of the parental antibody fragment.

In another embodiment, the conjugate has an effective size that is about 40 fold to about 100 fold greater, or is about 40 fold to about 80 fold greater, or is about 40 fold to about 50 fold greater, than the effective size of the parental antibody fragment.

In still another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW of at least about 20,000 D.

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In a further embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW of at least about 30,000 D.

In yet another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW of at least about 40,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 300,000 D, or is at or about 30,000 D to at or about 300,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 100,000 D, or is at or about 30,000 D to at or about 100,000 D, or is at or about 40,000 D to at or about 100,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 70,000 D, or is at or about 30,000 D to at or about 70,000 D, or is at or about 40,000 D to at or about 70,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 50,000 D, or is at or about 30,000 D to at or about 50,000 D, or is at or about 40,000 D to at or about 50,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one polymer having an actual MW that is at or about 20,000 D to at or about 40,000 D, or is at or about 30,000 D to at or about 40,000 D.

The conjugates of the invention can be made using any suitable technique now known or hereafter developed for derivatizing antibody fragments with polymers. It will be appreciated that the invention is not limited to conjugates utilizing any particular type of linkage between an antibody fragment and a polymer.

The conjugates of the invention include species wherein a polymer is covalently attached to a non-specific site or non-specific sites on the parental antibody fragment, i.e. polymer attachment is not targeted to a particular region or a particular amino acid residue in the parental antibody fragment. In such embodiments, the coupling chemistry can, for example, utilize the free epsilon amino groups of lysine residues in the parental antibody as attachment sites for the polymer, wherein such lysine residue amino groups are randomly derivatized with polymer.

In addition, the conjugates of the invention include species wherein a polymer is covalently attached to a specific site or specific sites on the parental antibody fragment, i.e. polymer attachment is targeted to a particular region or a particular amino acid residue or residues in the parental antibody fragment. In such embodiments, the coupling chemistry can, for example, utilize the free sulfhydryl group of a cysteine residue not in a disulfide bridge in the parental antibody fragment. In one embodiment, one or more cysteine residue(s) is (are) engineered into a selected site or sites in the parental antibody fragment for the purpose of providing a specific attachment site or sites for polymer. The polymer can be activated with any functional group that is capable of reacting specifically with the free sulfhydryl or thi 1 group(s) on the parental antibody, such as maleimide, sulfhydryl, thiol, triflate, tesylate, aziridine, exirane, and 5-pyridyl

functional groups. The polymer can be coupled to the parental antibody fragment using any protocol suitable for the chemistry of the coupling system selected, such as the protocols and systems described in Section (II)(1)(b) or in Section (T) of the Examples below.

In another embodiment, polymer attachment is targeted to the hinge region of the parental antibody fragment. The location of the hinge region varies according to the isotype of the parental antibody. Typically, the hinge region of IgG, IgD and IgA isotype heavy chains is contained in a proline rich peptide sequence extending between the C_H1 and C_H2 domains. In a preferred embodiment, a cysteine residue or residues is (are) engineered into the hinge region of the parental antibody fragment in order to couple polymer specifically to a selected location in the hinge region.

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In one aspect, the invention encompasses a conjugate having any molar ratio of polymer to antibody fragment that endows the conjugate with an apparent size in the desired range as taught herein. The apparent size of the conjugate will depend in part upon the size and shape of the polymer used, the size and shape of the antibody fragment used, the number of polymer molecules attached to the antibody fragment, and the location of such attachment site(s) on the antibody fragment. These parameters can easily be identified and maximized to obtain the a conjugate with the desired apparent size for any type of antibody fragment, polymer and linkage system.

In another aspect, the invention encompasses a conjugate with a polymer to antibody fragment molar ratio of no more than about 10:1, or no more than about 5:1, or no more than about 4:1, or no more than about 3:1, or no more than about 2:1, or no more than 1:1.

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In yet another aspect, the invention encompasses a conjugate wherein the antibody fragment is attached to about 10 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In another embodiment, the conjugate contains an antibody fragment attached to about 5 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In still another embodiment, the conjugate contains an antibody fragment attached to about 4 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In a further embodiment, the conjugate contains an antibody fragment attached to about 3 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 40,000 D. In an additional embodiment, the conjugate contains an antibody fragment attached to about 2 or fewer polymer molecules, each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. Also provided herein is a conjugate containing an antibody fragment attached to a single polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D.

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In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 300,000 D, or is at or about 30,000 D to at or about 300,000 D, and wherein the

conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 100,000 D, or is at or about 30,000 D to at or about 100,000 D, or is at or about 40,000 D to at or about 100,000 D, and wherein the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 70,000 D, or is at or about 30,000 D to at or about 70,000 D, or is at or about 40,000 D to at or about 70,000 D, and wherein the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 50,000 D, or is at or about 30,000 D to at or about 50,000 D, or is at or about 40,000 D to at or about 50,000 D, and wherein the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In still another aspect, the invention encompasses a conjugate wherein every polymer molecule in the conjugate has a molecular weight that is at or about 20,000 D to at or about 40,000 D, or is at or about 30,000 D to at or about 40,000 D, and wherein the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

It is believed that the serum half-life, MRT and/or serum clearance rate of any antibody fragment can be greatly improved by derivatizing the antibody fragment with polymer as taught herein. In one embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab',

Fab'-SH, Fv, scFv and F(ab')2.

In a preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer

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molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In yet another preferred embodiment, the conjugate contains a F(ab')₂ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In a further embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule and the polymer is coupled to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In an additional embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at least about 20,000 D in molecular weight, or at least about 40,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 300,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 4 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, every polymer molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, every polymer molecule in the conjugate is attached to the hinge region of the antibody fragment, and the conjugate contains no more than about 10 polymer molecules, or no more than about 5 polymer molecules, or no more than about 3 polymer molecules, or no more than about 2 polymer molecules, or no more than 1 polymer molecule.

In a further embodiment, the conjugate contains a F(ab')₂ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at least about 20,000 D in molecular weight, or at least about 30,000 D in molecular weight, or at least about 40,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains a F(ab')₂ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 300,000 D to at or about 300,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the

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corresponding cysteine residue in the opposite chain.

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In another embodiment, the conjugate contains a F(ab')₂ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains a F(ab')₂ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains a F(ab')₂ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains a F(ab')₂ antibody fragment attached to no more than about 2 polymer molecules, wherein every polymer molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, and wherein every polymer molecule in the conjugate is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In yet another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at least about 20,000 D in molecular weight, or at least about 30,000 D in molecular weight, or at least about 40,000 D in molecular weight, wherein the polymer

molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another embodiment, the conjugate contains an antibody fragment selected from the group

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consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, wherein the polymer molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In still another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at least about 20,000 D in molecular weight, or at least about 30,000 D in molecular weight, or at least about 40,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

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In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

In another embodiment, the conjugate contains an antibody fragment selected from the group

consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 polymer molecule, wherein the polymer molecule is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, and wherein the polymer molecule is attached to the hinge region of the antibody fragment.

Although any type of polymer is contemplated for use in constructing the conjugates of the invention, including the polymers and chemical linkage systems described in Section (II)(1)(b) below, polyethylene glycol (PEG) polymers are preferred for use herein.

In one embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW of at least about 20,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW of at least about 30,000 D.

In yet another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW of at least about 40,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 300,000 D, or is at or about 30,000 D to at or about 300,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 100,000 D, or is at or about 30,000 D to at or about 100,000 D, or is at or about 40,000 D to at or about 100,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 70,000 D, or is at or about 30,000 D to at or about 70,000 D, or is at or about 40,000 D to at or about 70,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 50,000 D, or is at or about 30,000 D to at or about 50,000 D, or is at or about 40,000 D to at or about 50,000 D.

In another embodiment, the conjugate is an antibody fragment covalently attached to at least one PEG having an actual MW that is at or about 20,000 D to at or about 40,000 D, or is at or about 30,000 D to at or about 40,000 D.

In another aspect, the invention encompasses a conjugate with a PEG to antibody fragment molar ratio of no more than about 10:1, or no more than about 5:1, or no more than about 4:1, or no more than about 3:1, or no more than about 2:1, or no more than 1:1.

In yet another aspect, the invention encompasses a conjugate wherein the antibody fragment is attached to about 10 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In another embodiment, the conjugate contains an antibody fragment attached to about 5 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In still another embodiment, the conjugate contains an antibody fragment attached to about 4 or fewer PEG

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molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In a further embodiment, the conjugate contains an antibody fragment attached to about 3 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In an additional embodiment, the conjugate contains an antibody fragment attached to about 2 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. Also provided herein is a conjugate containing an antibody fragment attached to a single PEG molecule having a molecular weight of at least about 20,000 D, or at least about 40,000 D.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 300,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecules.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecules.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate wherein the antibody fragment is

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derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In still another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')2, wherein the antibody fragment is attached to about 10 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In another embodiment, the foregoing conjugate contains an antibody fragment attached to about 5 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In still another embodiment, the foregoing conjugate contains an antibody fragment attached to about 4 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In a further embodiment, the foregoing conjugate contains an antibody fragment attached to about 3 or fewer PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. In an additional embodiment, the foregoing conjugate contains an antibody fragment attached to about 2 or few r PEG molecules, each PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. Also provided herein is the foregoing conjugate that contains an antibody fragment attached to a single PEG molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG

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In-another-aspect, the-invention-encompasses a conjugate containing an antib dy fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another aspect, the invention encompasses a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH and F(ab')₂, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 2 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In a preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight of at least about 20,000D, or at least about 30,000D, or at least about 40,000D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight that is at or about 20,000 D to about 300,000 D, or is at or about 30,000 D to at or about 300,000 D, or is at or about 40,000 D to at or about 300,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight that is at or about 20,000 D to about 100,000 D, or is at or about 30,000 D to at or about

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100,000 D, or is at or about 40,000 D to at or about 100,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight that is at or about 20,000 D to about 70,000 D, or is at or about 30,000 D to at or about 70,000 D, or is at or about 40,000 D to at or about 70,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight that is at or about 20,000 D to about 50,000 D, or is at or about 30,000 D to at or about 50,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG having a molecular weight that is at or about 20,000 D to about 40,000 D, or is at or about 30,000 D to at or about 40,000 D, and wherein every PEG molecule in the conjugate is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at least about 20,000D in molecular weight, or at least about 30,000D in molecular weight, or at least about 40,000D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular

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weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecule.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, wherein every PEG molecule in the conjugate molecule is attached to the hinge region of the antibody fragment, and wherein the conjugate contains no more than about 10 PEG molecules, or no more than about 5 PEG molecules, or no more than about 4 PEG molecules, or no more than about 3 PEG molecules, or no more than about 2 PEG molecules, or no more than 1 PEG molecules.

In yet another preferred embodiment, the conjugate contains a F(ab')₂ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at least about 20,000D in molecular weight, or at least about 30,000D in molecular weight, or at least about 40,000D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would

ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains a F(ab')₂ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, or is at or about 40,000 D to at or about 300,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains a F(ab')₂ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, or is at or about 40,000 D to at or about 100,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains a F(ab')₂ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains a F(ab')₂ antibody fragment derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains a F(ab')2 antibody fragment derivatized

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with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular—weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, wherein the antibody fragment is attached to no more than about 2 PEG molecules, and wherein every PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In still another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at least about 20,000 D in molecular weight, or at least about 30,000 in molecular weight, or at least about 40,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 300,000 D in molecular weight, or is at or about 30,000 D to at or about 300,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 100,000 D in molecular weight, or is at or about 30,000 D to at or about 100,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 70,000 D in molecular weight, or is at or about 30,000 D to at or about 70,000 D in molecular weight, or is at or about 40,000 D to at or about 70,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG

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molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 50,000 D in molecular weight, or is at or about 30,000 D to at or about 50,000 D in molecular weight, or is at or about 40,000 D to at or about 50,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is derivatized with PEG, wherein every PEG molecule in the conjugate is at or about 20,000 D to at or about 40,000 D in molecular weight, or is at or about 30,000 D to at or about 40,000 D in molecular weight, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is attached to a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains, wherein the disulfide bridge is avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposite chain.

It will be appreciated that all of the above-described embodiments of the invention utilizing PEG polymers include conjugates wherein the PEG polymer(s) is (are) linear or branched. In a preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is branched and at least about 40,000 D in molecular weight. In a particularly surprising and unexpected finding, the inventors discovered that the foregoing conjugate exhibits a serum half-life, MRT and serum clearance rate approaching that of full length antibody as shown in Example X below.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 300,000 D.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 100,000 D.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the

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group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1-PEG-molecule, and-wherein-the-PEG-molecule-is-branched-and-has-a-molecular_weight_that_is_at_or_about 40,000 D to at or about 70,000 D.

In another preferred embodiment, the conjugate contains an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, and wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 50,000 D.

In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and at least 40,000D in molecular weight, and the PEG molecule is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 300,000 D, and the PEG molecule is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 100,000 D, and the PEG molecule is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 70,000 D, and the PEG molecule is attached to the hinge region of the antibody fragment.

In another preferred embodiment, the invention provides a conjugate containing an antibody fragment selected from the group consisting of Fab, Fab', and Fab'-SH, wherein the antibody fragment is attached to no more than 1 PEG molecule, wherein the PEG molecule is branched and has a molecular weight that is at or about 40,000 D to at or about 50,000 D, and the PEG molecule is attached to the hinge region of the antibody fragment.

In one aspect, the invention provides any of the above-described conjugates wherein the conjugate contains no more than one antibody fragment. Additionally provided herein is any of the above-described conjugates wherein the conjugate contains one or more antibody fragment(s) covalently linked to one or more polymer molecule(s), such as conjugates containing two or more antibody fragments covalently linked together by polymer molecule(s). In one embodiment, a polymer molecule is used to link together two antibody fragments to form a dumbbell-shaped structure. Also encompassed herein are conjugates formed

by more than two antibody fragments joined by polymer molecule(s) to form a rosette or other shapes. The antibody fragments in such structures can be of the same or different fragment type and can have the same antigen specificity or have different antigen specificities. Such structures can be made by using a polymer molecule derivatized with multiple functional groups permitting the direct attachment, or the attachment by means of bi- or multi-functional linkers, of two or more antibody fragments to the polymer backbone.

In another aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising an antigen recognition site that binds to rabbit IL-8 and/or human IL-8. In yet another aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising 6G4.2.5LV/L1N35A or 6G4.2.5LV/L1N35E as defined below. In still another aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising 6G4.5.2.5HV11 as defined below. In a further aspect, the invention encompasses any of the abovedescribed conjugates utilizing an antibody fragment comprising hu6G4.2.5LV/L1N35A or hu6G4.2.5LV/L1N35E as defined below. In an additional aspect, the invention encompasses any of the above-described conjugates utilizing an antibody fragment comprising hu6G4.2.5HV. Further encompassed herein are any of the above-described conjugates utilizing an antibody fragment comprising 6G4.2.5LV/L1N35A or 6G4.2.5LV/L1N35E and further comprising the CDRs of 6G4.2.5HV as defined below. Also encompassed herein are any of the above described conjugates utilizing an antibody fragment comprising hu6G4.2.5LV/L1N35A or hu6G4.2.5LV/L1N35E and further comprising hu6G4.2.5HV as defined below. Additionally encompassed herein are any of the above-described conjugates utilizing an antibody fragment comprising 6G4.2.5LV11N35A or 6G4.2.5LV11N35E as defined below. Further provided herein are any of the above-described conjugates utilizing an antibody fragment comprising 6G4.2.5LV11N35A or 6G4.2.5LV11N35E and further comprising 6G4.2.5HV11 as defined below.

a. Production of Antibody Fragments

Antibody fragments can be produced by any method known in the art. Generally, an antibody fragment is derived from a parental intact antibody. The parental antibody can be generated by raising polyclonal sera against the desired antigen by multiple subcutaneous (sc) or intraperitoneal (ip) injections of antigen and an adjuvant, such as monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, MT), at multiple sites. Two weeks later the animals are boosted. 7 to 14 days later animals are bled and the serum is assayed for anti-antigen titer. Animals are boosted until titer plateaus. Sera are harvested from animals, and polyclonal antibodies are isolated from sera by conventional immunoglobulin purification procedures, such as protein A-Sepharose chromatography, hydroxylapatite chromatography, gel filtration, dialysis, or antigen affinity chromatography. The desired antibody fragments can be generated from purified polyclonal antibody preparations by conventional enzymatic methods, e.g. F(ab')₂ fragments are produced by pepsin cleavage of intact antibody, and Fab fragments are produced by briefly digesting intact antibody with papain.

Alternatively, antibody fragments are derived from monoclonal antibodies generated against the desired antigen. Monoclonal antibodies may be made using the hybridoma method first described by Kohler

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et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOP-21 and M.C.-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., Anal. Biochem., 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

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DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of antibody-encoding DNA include Skerra et al., Curr. Opinion in Immunol., 5: 256 (1993) and Pluckthun, Immunol. Revs., 130: 151 (1992).

In a preferred embodiment, the antibody fragment is derived from a humanized antibody. Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. It will be appreciated that variable domain sequences obtained from any non-human animal phage display library-derived Fv clone or from any non-human animal hybridoma-derived antibody clone provided as described herein can serve as the "import" variable domain used in the construction of the humanized antibodies of the invention. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321: 522 (1986); Riechmann et al., Nature, 332: 323 (1988); Verhoeyen et al., Science, 239: 1534 (1988)), by substituting non-human animal, e.g. rodent, CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly et al., supra), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in non-human animal, e.g. rodent, antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a non-human animal, e.g. rodent, antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the non-human animal is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol., 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol., 196: 901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup light or heavy chains. The same framework can be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci USA, 89: 4285 (1992); Presta et al., J. Immunol., 151: 2623 (1993)).

It is also important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional

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immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs-are-available-which-illustrate-and-display-probable-three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind to its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

In addition, antibody fragments for use herein can be derived from human monoclonal antibodies. Human monoclonal antibodies against the antigen of interest can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).

It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. Acad. Sci USA, 90: 2551 (1993); Jakobovits et al., Nature, 362: 255 (1993); Bruggermann et al., Year in Immunol., 7: 33 (1993).

Alternatively, phage display technology (McCafferty et al., Nature 348:552 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned inframe into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson et al., Current Opinion in Structural Biology 3:564 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature 352:624 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol. 222:581 (1991), or Griffith et al., EMBO J. 12:725 (1993).

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In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al., Bio/Technol. 10:779 (1992)). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse et al., Nucl. Acids Res. 21:2265 (1993).

Gene shuffling can also be used to derive human antibodies from non-human, e.g. rodent, antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called "epitope imprinting", either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described above is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone, i.e. the epitope governs (imprints) the choice of the human chain partner. When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213 published April 1, 1993). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin.

The invention also encompasses the use of bispecific and heteroconjugate antibody fragments having specificities for at least two different antigens. Bispecific and heteroconjugate antibodies can be prepared as full length antibodies or as antibody fragments (e.g. F(ab')₂ bispecific antibody fragments). Antibody fragments having more than two valencies (e.g. trivalent or higher valency antibody fragments) are also contemplated for use herein. Bispecific antibodies, heteroconjugate antibodies, and multi-valent antibodies can be prepared as described in Section (II)(3)(C) below.

As described above, DNA encoding the monoclonal antibody or antibody fragment of interest can be isolated from its hybridoma or phage display clone of origin, and then manipulated to create humanized and/or affinity matured constructs. In addition, known techniques can be employed to introduce an amino acid residue or residues into any desired location on the polypeptide backbone of the antibody fragment, e.g. a cysteine residue placed in the hinge region of the heavy chain, thereby providing a site for specific attachment of polymer molecule(s). In one embodiment, the native cysteine residue in either the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains is substituted with another amino acid, such as serine, in order to leave the partner cysteine residue in the opposite chain with a free suflhydryl for specific attachment of polymer molecule.

Upon construction of the desired antibody or antibody fragment-encoding clone, the clone can be used for recombinant production of the antibody fragment as described in Section (II)(4) below. Finally, the antibody or antibody fragment product can be recovered from host cell culture and purified as described in Section (II)(4)(F) below. In the case of embodiments utilizing an antibody fragment engineered to lack a cysteine residue that ordinarily forms the disulfide bridge between the light and heavy chains as described above, preferred recombinant production systems include bacterial expression and product recovery procedures utilizing the low pH osmotic shock method described in the "Alternative Fab'-SH Purification" section of Example T below. If a full length antibody is produced, the desired antibody fragment can be obtained therefrom by subjecting the intact antibody to enzymatic digestion according to known methods, e.g. as described in Section (II)(4)(G) below.

b. Construction of Antibody Fragment-Polymer Conjugates

The antibody fragment-polymer conjugates of the invention can be made by derivatizing the desired antibody fragment with an inert polymer. It will be appreciated that any inert polymer which provides the conjugate with the desired apparent size or which has the selected actual MW as taught herein is suitable for use in constructing the antibody fragment-polymer conjugates of the invention.

Many inert polymers are suitable for use in pharmaceuticals. See, e.g., Davis et al., Biomedical Polymers: Polymeric Materials and Pharmaceuticals for Biomedical Use, pp.441-451 (1980). In all embodiments of the invention, a non-proteinaceous polymer is used. The nonproteinaceous polymer ordinarily is a hydrophilic synthetic polymer, i.e., a polymer not otherwise found in nature. However, polymers which exist in nature and are produced by recombinant or in vitro methods are also useful, as are polymers which are isolated from native sources. Hydrophilic polyvinyl polymers fall within the scope of this invention, e.g. polyvinylalcohol and polyvinylpyrrolidone. Particularly useful are polyalkylene ethers such as polyethylene glycol (PEG); polyoxyalkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene (Pluronics); polymethacrylates; carbomers; branched or unbranched polysaccharides which comprise the saccharide monomers D-mannose, D- and Lgalactose, fucose, fructose, D-xylose, L-arabinose, D-glucuronic acid, sialic acid, D-galacturonic acid, Dmannuronic acid (e.g. polymannuronic acid, or alginic acid), D-glucosamine, D-galactosamine, D-glucose and neuraminic acid including homopolysaccharides and heteropolysaccharides such as lact se, amylopectin, starch, hydroxyethyl starch, amylose, dextrane sulfate, dextran, dextrins, glycogen, or the polysaccharide subunit of acid mucopolysaccharides, e.g. hyaluronic acid; polymers of sugar alcohols such as polysorbitol and polymannitol; heparin or heparon. The polymer prior to cross-linking need not be, but preferably is, water soluble, but the final conjugate must be water soluble. Preferably, the conjugate exhibits a water solubility of at least about 0.01 mg/ml, and more preferably at least about 0.1 mg/ml, and still more preferably at least about 1 mg/ml. In addition, the polymer should not be highly immunogenic in the conjugate form, nor should it possess viscosity that is incompatible with intravenous infusion or injection if the conjugate is intended to be administered by such routes.

In one embodiment, the polymer contains only a single group which is reactive. This helps to

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avoid cross-linking of protein molecules. However, it is within the scope herein to maximize reaction conditions to reduce cross-linking, or to purify the reaction products through gel filtration or ion exchange chromatography to recover substantially homogenous derivatives. In other embodiments, the polymer contains two or more reactive groups for the purpose of linking multiple antibody fragments to the polymer backbone. Again, gel filtration or ion exchange chromatography can be used to recover the desired derivative in substantially homogeneous form.

The molecular weight of the polymer can range up to about 500,000 D, and preferably is at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D. The molecular weight chosen can depend upon the effective size of the conjugate to be achieved, the nature (e.g. structure, such as linear or branched) of the polymer, and the degree of derivatization, i.e. the number of polymer molecules per antibody fragment, and the polymer attachment site or sites on the antibody fragment.

The polymer can be covalently linked to the antibody fragment through a multifunctional crosslinking agent which reacts with the polymer and one or more amino acid residues of the antibody fragment to be linked. However, it is also within the scope of the invention to directly crosslink the polymer by reacting a derivatized polymer with the antibody fragment, or vice versa.

The covalent crosslinking site on the antibody fragment includes the N-terminal amino group and epsilon amino groups found on lysine residues, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. The polymer may be covalently bonded directly to the antibody fragment without the use of a multifunctional (ordinarily bifunctional) crosslinking agent. Covalent binding to amino groups is accomplished by known chemistries based upon cyanuric chloride, carbonyl diimidazole, aldehyde reactive groups (PEG alkoxide plus diethyl acetal of bromoacetaldehyde; PEG plus DMSO and acetic anhydride, or PEG chloride plus the phenoxide of 4-hydroxybenzaldehyde, activated succinimidyl esters, activated dithiocarbonate PEG, 2,4,5-trichlorophenylcloroformate or P-nitrophenylcloroformate activated PEG.) Carboxyl groups are derivatized by coupling PEG-amine using carbodiimide. Sulfhydryl groups are derivatized by coupling to maleimido-substituted PEG (e.g. alkoxy-PEG amine plus sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) as described in WO 97/10847 published March 27, 1997, or PEG-maleimide commercially available from Shearwater Polymers, Inc., Huntsville, AL). Alternatively, free amino groups on the antibody fragment (e.g. epsilon amino groups on lysine residues) can be thiolated with 2-imino-thiolane (Traut's reagent) and then coupled to maleimide-containing derivatives of PEG as described in Pedley et al., Br. J. Cancer, 70: 1126-1130 (1994).

The polymer will bear a group which is directly reactive with an amino acid side chain, or the N- or C-terminus of the polypeptide linked, or which is reactive with the multifunctional cross-linking agent. In general, polymers bearing such reactive groups are known for the preparation of immobilized proteins. In order to use such chemistries here, one should employ a water soluble polymer otherwise derivatized in the same fashion as insoluble polymers heretofore employed for protein immobilization. Cyanogen bromide activation is a particularly useful procedure to employ in crosslinking polysaccharides.

"Water soluble" in reference to the starting polymer means that the polymer or its reactive

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intermediate used for conjugation is sufficiently water soluble to participate in a derivatization reaction.

The degree of substitution with such a polymer will vary depending upon the number of reactive sites on the antibody fragment, the molecular weight, hydrophilicity and other characteristics of the polymer, and the particular antibody fragment derivatization sites chosen. In general, the conjugate contains from 1 to about 10 polymer molecules, but greater numbers of polymer molecules attached to the antibody fragments of the invention are also contemplated. The desired amount of derivatization is easily achieved by using an experimental matrix in which the time, temperature and other reaction conditions are varied to change the degree of substitution, after which the level of polymer substitution of the conjugates is determined by size exclusion chromatography or other means known in the art.

The polymer, e.g. PEG, is cross-linked to the antibody fragment by a wide variety of methods known per se for the covalent modification of proteins with nonproteinaceous polymers such as PEG. Certain of these methods, however, are not preferred for the purposes herein. Cyanuronic chloride chemistry leads to many side reactions, including protein cross-linking. In addition, it may be particularly likely to lead to inactivation of proteins containing sulfhydryl groups. Carbonyl diimidazole chemistry (Beauchamp et al., Anal Biochem. 131, 25-33 [1983]) requires high pH (>8.5), which can inactivate proteins. Moreover, since the "activated PEG" intermediate can react with water, a very large molar excess of "activated PEG" over protein is required. The high concentrations of PEG required for the carbonyl diimidazole chemistry also led to problems in purification, as both gel filtration chromatography and hydrophilic interaction chromatography are adversely affected. In addition, the high concentrations of "activated PEG" may precipitate protein, a problem that per se has been noted previously (Davis, U.S. Patent No. 4,179,337). On the other hand, aldehyde chemistry (Royer, U.S. Patent No. 4,002,531) is more efficient since it requires only a 40-fold molar excess of PEG and a 1-2 hr incubation. However, the manganese dioxide suggested by Royer for preparation of the PEG aldehyde is problematic "because of the pronounced tendency of PEG to form complexes with metal-based oxidizing agents" (Harris et al., J. Polym. Sci. Polym. Chem. Ed. 22, 341-52 [1984]). The use of a Moffatt oxidation, utilizing DMSO and acetic anhydride, obviates this problem. In addition, the sodium borohydride suggested by Royer must be used at high pH and has a significant tendency to reduce disulfide bonds. In contrast, sodium cyanoborohydride, which is effective at neutral pH and has very little tendency to reduce disulfide bonds is preferred. In another preferred embodiment, maleimido-activated PEG is used for coupling to free thiols on the antibody fragment.

Functionalized PEG polymers to modify the antibody fragments of the invention are available from Shearwater Polymers, Inc. (Huntsville, AL). Such commercially available PEG derivatives include, but are not limited to, amino-PEG, PEG amino acid esters, PEG-hydrazide, PEG-thiol, PEG-succinate, carboxymethylated PEG, PEG-propionic acid, PEG amino acids, PEG succinimidyl succinate, PEG succinimidyl propionate, succinimidyl ester of carboxymethylated PEG, succinimidyl carbonate of PEG, succinimidyl esters of amino acid PEGs, PEG-oxycarbonylimidazole, PEG-nitrophenyl carbonate, PEG tresylate, PEG-glycidyl ether, PEG-aldehyde, PEG vinylsulfone, PEG-maleimide, PEG-orthopyridyl-

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disulfide, heterofunctional PEGs, PEG vinyl derivatives, PEG silanes, and PEG phospholides. The reaction conditions for coupling these PEG derivatives will vary depending on the protein, the desired degree of PEGylation, and the PEG derivative utilized. Some factors involved in the choice of PEG derivatives include: the desired point of attachment (such as lysine or cysteine R-groups), hydrolytic stability and reactivity of the derivatives, stability, toxicity and antigenicity of the linkage, suitability for analysis, etc. Specific instructions for the use of any particular derivative are available from the manufacturer.

The conjugates of this invention are separated from the unreacted starting materials by gel filtration or ion exchange HPLC. Heterologous species of the conjugates are purified from one another in the same fashion.

The conjugates may also be purified by ion-exchange chromatography. The chemistry of many of the electrophilically activated PEG's results in a reduction of amino group charge of the PEGylated product. Thus, high resolution ion exchange chromatography can be used to separate the free and conjugated proteins, and to resolve species with different levels of PEGylation. In fact, the resolution of different species (e.g. containing one or two PEG residues) is also possible due to the difference in the ionic properties of the unreacted amino acids. In one embodiment, species with difference levels of PEGylation are resolved according to the methods described in WO 96/34015 (International Application No. PCT/US96/05550 published October 31, 1996).

In a preferred embodiment, the conjugate is generated by utilizing the derivatization and purification methods described in Section (T) of the Examples below.

In one aspect, the invention provides any of the above-described conjugates formed by its component parts, i.e. one or more antibody fragment(s) covalently attached to one or more polymer molecule(s), without any extraneous matter in the covalent molecular structure of the conjugate.

c. Other Derivatives of Large Effective Size Conjugates

In another aspect, any of the above-described conjugates can be modified to contain one or more component(s) in addition to the antibody fragment component(s) and polymer component(s) that form the conjugate, wherein the modification does not alter the essential functional property of the conjugate, namely, the substantially improved serum half-life, MRT and/or serum clearance rate as compared to that of the parental antibody fragment from which the conjugate is derived. In one embodiment, the invention provides any of the above-described conjugates modified to incorporate one or more nonproteinaceous functional group(s). For example, the conjugate can be modified to incorporate nonproteinaceous labels or reporter molecules, such as radiolabels, including any radioactive substance used in medical treatment or imaging or used as an effector function or tracer in an animal model, such as radioisotopic labels ⁹⁹Tc, ⁹⁰Y, ¹¹¹In, ³²P, ¹⁴C, ¹²⁵I, ³H, ¹³¹I, ¹¹C, ¹⁵O, ¹³N, ¹⁸F, ³⁵S, ⁵¹Cr, ⁵⁷To, ²²⁶Ra, ⁶⁰Co, ⁵⁹Fe, ⁷⁵Se, ¹⁵²Eu, ⁶⁷Cu, ²¹⁷Ci, ²¹¹At, ²¹²Pb, ⁴⁷Sc, ¹⁰⁹Pd, ²³⁴Th, ⁴⁰K, and the like, non-radioisotopic labels such as ¹⁵⁷Gd, ⁵⁵Mn, ⁵²Tr, ⁵⁶Fe, etc., fluroescent or chemiluminescent labels, including fluorophores such as rare earth chelates, fluorescein and its derivatives, rhodamine and its derivatives, isothiocyanate, phycoerythrin, phycocyanin,

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allophycocyanin, o-phthaladehyde, fluorescamine, ¹⁵²Eu, dansyl, umbelliferone, luciferin, luminal label, isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridimium salt-label, an oxalate-ester label, an aequorin label, 2,3-dihydrophthalazinediones, biotin/avidin, spin labels, stable free radicals, and the like.

Conventional methods are available to bind these labels covalently to the polypeptide antibody fragment or polymer component of the conjugate. In one aspect, any conjugate of the invention is modified by derivatizing the antibody fragment component with any of the above-described non-proteinaceous labels, wherein the label is directly or indirectly (through a coupling agent) attached to the antibody fragment, and wherein such derivatization of the antibody fragment does not contribute or introduce any polymer moiety into the molecular structure of the conjugate. For instance, coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine, and the like can be used to tag the antibody fragment with the above-described fluorescent or chemiluminescent labels. See, for example, U.S. Pat. No. 3,940,475 (fluorimetry), Morrison, Meth. Enzymol., 32b, 103 (1974), Svyanen et al., J. Biol. Chem., 284, 3762 (1973), and Bolton and Hunter, Biochem. J., 133, 529 (1973).

In the case of embodiments utilizing radiolabels, both direct and indirect labeling can be used to incorporate the selected radionuclide into the conjugate. As used herein in the context of radiolabeling, the phrases "indirect labeling" and "indirect labeling approach" both mean that a chelating agent is covalently attached to the antibody fragment moiety or polymer moiety of the conjugate and at least one raidonuclide is inserted into the chelating agent. Preferred chelating agents and radionuclides are set forth in Srivagtava, S.C. and Mease, R.C., "Progress in Research on Ligands, Nuclides and Techniques for Labeling Monoclonal Antibodies," Nucl. Med. Bio., 18(6): 589-603 (1991). A particularly preferred chelating agent is 1isothiocycmatobenzyl-3-methyldiothelene triaminepent acetic acid ("MX-DTPA"). As used herein in the context of radiolabeling, the phrases "direct labeling" and "direct labeling approach" both mean that a radionuclide is covalently attached directly to the antibody fragment moiety (typically via an amino acid residue) or to the polymer moiety of the conjugate. Preferred radionuclides for use in direct labeling of conjugate are provided in Srivagtava and Mease, supra. In one embodiment, the conjugate is directly labeled with 131 covalently attached to tyrosine residues. In another embodiment, the antibody fragment component of the conjugate is directly or indirectly labeled with any of the above-described radiolabels, wherein such labeling of the antibody fragment does not contribute or introduce any polymer moiety into the molecular structure of the conjugate.

d. Therapeutic Compositions and Administration of Large Effective Size Conjugates

The conjugate of the invention is useful for treating the disease indications that are treated with the parent intact antibody. For example, a conjugate derived from an anti-IL-8 antibody or fragment is useful in the treatment of inflammatory disorders as described in Section (II)(5)(B) below. Therapeutic formulations of the conjugate of the invention can be prepared by utilizing the same procedures described for the formulation of the anti-IL-8 antibodies and fragments of the invention in Section (II)(5)(B) below. The conjugate of the invention can be administered in place of the parent antibody for a given disease indication

by modifying the formulation, dosage, administration protocol, and other aspects of a therapeutic regimen as required by the different pharmacodynamic characteristics of the conjugate and as dictated by common medical knowledge and practice.

e. Reagent Uses for Large Effective Size Conjugates

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The conjugate of the invention also finds application as a reagent in an animal model system for in vivo study of the biological functions of the antigen recognized by the conjugate. The conjugate would enable the practitioner to inactivate or detect the cognate antigen in circulation or in tissue for a far greater period of time than would be possible with art-known constructs while removing any Fc interaction (which could attend the use of an intact antibody) from the system. In addition, the increased half-life of the conjugate of the invention can be applied advantageously to the induction of tolerance for the underivatized antibody fragment in a test animal by employing the Wie et al., Int. Archs. Allergy Appl. Immunol., 64: 84-99 (1981) method for allergen tolerization, which would permit the practitioner to repeatedly challenge the tolerized animal with the underivatized parental antibody fragment without generating an immune response against the parental fragment.

2. HUMANIZED 6G4.2.5 MONOCLONAL ANTIBODIES AND ANTIBODY FRAGMENTS

In one embodiment, the invention provides an antibody fragment or full length antibody comprising a heavy chain comprising the amino acid sequence of amino acids 1-230 (herein referred to as "6G4.2.5HV11") of the humanized anti-IL-8 6G4.2.5v11 heavy chain polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75).

The invention encompasses a single chain antibody fragment comprising the 6G4.2.5HV11, with or without any additional amino acid sequence. In one embodiment, the invention provides a single chain antibody fragment comprising the 6G4.2.5HV11 without any associated light chain amino acid sequence, i.e. a single chain species that makes up one half of a Fab fragment.

Further provided herein are an antibody or antibody fragment comprising the 6G4.2.5HV11, and further comprising a light chain comprising the amino acid sequence of amino acids 1-219 (herein referred to as "6G4.2.5LV11") of the humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65).

In one embodiment, the invention provides a single chain antibody fragment wherein the 6G4.2.5HV11 and the 6G4.2.5LV11 are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment comprises the 6G4.2.5HV11 joined to the 6G4.2.5LV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising the 6G4.2.5HV11 joined to the 6G4.2.5LV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the 6G4.2.5HV11 and a second polypeptide

chain comprises the 6G4.2.5LV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the foregoing two-chain antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, and F(ab')₂.

The invention also provides an antibody or antibody fragment comprising a heavy chain containing the 6G4.2.5HV11 and optionally further comprising a light chain containing the 6G4.2.5LV11, wherein the heavy chain, and optionally the light chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al. (supra).

In a preferred embodiment, the antibody or antibody fragment comprises the 6G4.2.5HV11 in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity and/or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney et al., J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below. In a preferred embodiment, the antibody or antibody fragment comprises the 6G4.2.5HV11 fused at its C-terminus to the GCN4 leucine zipper to yield the amino acid sequence of amino acids 1-275 (herein referred to as "6G4.2.5HV11GCN4") of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75).

3. <u>VARIANTS OF HUMANIZED 6G4.2.5 MONOCLONAL ANTIBODIES AND ANTIBODY</u> FRAGMENTS

The invention additionally encompasses humanized anti-IL-8 monoclonal antibody and antibody fragments comprising variants of the 6G4.2.5 complementarity determining regions (CDRs) or variants of the 6G4.2.5v11 variable domains which exhibit higher affinity for human IL-8 and/or possess properties that yield greater efficiency in recombinant production processes.

A. 6G4.2.5LV VARIANTS

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In one aspect, the invention provides humanized anti-IL-8 monoclonal antibodies and antibody fragments comprising the complementarity determining regions (referred to herein as the "CDRs of 6G4.2.5LV") L1, L2, and L3 of the 6G4.2.5 light chain variable domain amino acid sequence of Fig. 24, wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

In addition, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a humanized light chain variable domain comprising a variant (hereinafter referred to a "6G4.2.5LV CDRs variant") of the complementarity determining regions L1, L2, and L3 of the 6G4.2.5 variable light chain domain amino acid sequence of Fig. 24 (SEQ ID NO: 48). In one embodiment, the

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invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35X35") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Asn (denoted as "X35") is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48). In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48). In another preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35E") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Glu is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

In a second aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26X₂₆") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48). In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Ser at amino acid position 26, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

In a third aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L3H98X₉₈") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98. In a

preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L3H98A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid position 98.

In a fourth aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26X₂₆,N35X₃₅") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26 and any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48). In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26A,N35A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Ser at amino acid position 26 and Ala is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

In a fifth aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35X₃₅/L3H98X₉₈") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98. In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1N35A/L3H98A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid position 98.

In a sixth aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26X₂₆/L3H98X_{QR}")

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wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98. In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (herein referred to as "6G4.2.5LV/L1S26A/L3H98A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Ser at amino acid position 26, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid position 98.

In a seventh aspect, the invention provides a variant 6G4.2.5 humanized antibody or antibody 6G4.2.5LV CDRs variant (here referred comprising fragment "6G4.2.5LV/L1S26X₂₆,N35X₃₅/L3H98X₉₈") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than Ser (denoted as " X_{26} ") is substituted for Ser at amino acid position 26 and any amino acid other than Asn (denoted as " X_{35} ") is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that any amino acid other than His (denoted as "X₉₈") is substituted for His at amino acid position 98. In a preferred embodiment, the invention provides a variant 6G4.2.5 humanized antibody or antibody fragment comprising a 6G4.2.5LV CDRs variant (here referred to as "6G4.2.5LV/L1S26A,N35A/L3H98A") wherein L1 corresponds to amino acids 24-39 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for Ser at amino acid position 26 and Ala is substituted for Asn at amino acid position 35, L2 corresponds to amino acids 55-61 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48), and L3 corresponds to amino acids 94-102 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48) with the proviso that Ala is substituted for His at amino acid position 98.

The humanized light chain variable domains of the invention can be constructed by using any of the techniques for antibody humanization known in the art. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature 321:522 (1986); Riechmann et al., Nature 332:323 (1988); Verhoeyen et al., Science 239:1534 (1988)), by substituting the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant for the corresponding sequences of a human antibody light chain variable domain. Accordingly, such "humanized" derivatives containing the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5VL CDRs variant are chimeric (Cabilly et al., supra). The humanized

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light chain variable domain comprising the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant can also contain some FR residues that are substituted by residues from analogous sites in the murine 6G4.2.5 antibody light chain variable domain ("6G4.2.5LV"). The complete amino acid sequence of 6G4.2.5LV is set out as amino acids 1-114 of the amino acid sequence of Fig. 24 (SEQ ID NO: 48).

The invention further provides a humanized antibody or antibody fragment comprising a humanized light chain variable domain comprising the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant as described above, and further comprising a humanized heavy chain variable domain comprising the complementarity determining regions (CDRs) H1, H2, and H3 of the 6G4.2.5 (murine monoclonal antibody) variable heavy chain domain amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). The above-described H1, H2, and H3 CDRs of the 6G4.2.5 heavy chain variable domain ("6G4.2.5HV") are collectively referred to as the "CDRs of 6G4.2.5HV".

In another embodiment, the invention provides a humanized antibody or antibody fragment comprising a humanized light chain variable domain comprising the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant as described above, and further comprising a humanized heavy chain variable domain comprising a variant (herein referred to as a "6G4.2.5HV CDRs variant") of the H1, H2, and H3 CDRs of the 6G4.2.5 (murine monoclonal antibody) variable heavy chain domain amino acid sequence of Fig. 25 (SEQ ID NO: 50). In one 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). With the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50).

In a second 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). H2 corresponds to amino acids 50-66 of the

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amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50).

In a third 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D100E"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100.

In a fourth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3R102K"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

In a fifth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D106E"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

In a seventh 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D100E,R102K"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102.

In an eighth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3R102K,D106E"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

In a ninth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D100E,D106E"), wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

In a tenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H3D100E,R102K,D106E"),

wherein H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), wherein H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and wherein H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102, and Glu is substituted for Asp at amino acid position 106.

6G4.2.5HV **CDRs** variant (referred In an eleventh to herein as "6G4.2.5HV/H1S31Z31/H2S54Z54"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50). In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50).

In a twelfth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3D100E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D100E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100.

In a thirteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3R102K"), H1

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correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

A fourteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

herein as (referred variant fifteenth 6G4.2.5HV **CDRs** Α "6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D100E,R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102.

In a sixteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H3R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position

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102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

herein **CDRs** variant (referred as In а seventeenth 6G4.2.5HV "6G4.2.5HV/H1S31Z31/H3D100E,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H3D100E,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106.

(referred herein eighteenth 6G4.2.5HV **CDRs** variant to as In an "6G4.2.5HV/H1S31Z31/H3D100E,R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z31") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid In a preferred 6G4.2.5HV CDRs variant (referred position 106. "6G4.2.5HV/H1S31A/H3D100E,R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

In a nineteenth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3D100E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of

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Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D100E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100.

In a twentieth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3R102K"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3R102K"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

(referred herein to variant 6G4.2.5HV **CDRs** twenty-first "6G4.2.5HV/H2S54Z₅₄/H3D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

In a twenty-second 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is

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substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D100E,R102K"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102.

6G4.2.5HV **CDRs** variant (referred herein as In twenty-third "6G4.2.5HV/H2S54Z₅₄/H3R102K,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3R102K,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

herein twenty-fourth **CDRs** variant (referred to In 6G4.2.5HV "6G4.2.5HV/H2S54Z₅₄/H3D100E,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H2S54A/H3D100E,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106.

In a twenty-fifth 6G4.2.5HV CDRs variant (referred to herein as

"6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid (referred to herein CDRs variant preferred 6G4.2.5HV position ln "6G4.2.5HV/H2S54A/H3D100E,R102K,D106E"), H1 corresponds to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50), H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

twenty-sixth 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino In a preferred 6G4.2.5HV CDRs variant (referred to herein as acid position 100. "6G4.2.5HV/H1S31A/H2S54A/H3D100E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100.

In a twenty-seventh 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino

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acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102.

herein as (referred **CDRs** variant In twenty-eighth 6G4.2.5HV " $6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}/H3D106E$ "), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino preferred 6G4.2.5HV CDRs variant (referred to herein position 106. acid "6G4.2.5HV/H1S31A/H2S54A/H3D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 106.

herein 6G4.2.5HV **CDRs** variant (referred to twenty-ninth In "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino acid position 102. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Lys is substituted for Arg at amino

acid position 102.

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CDRs variant (referred herein as 6G4.2.5HV thirtieth In "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

herein (referred **CDRs** variant ln thirty-first 6G4.2.5HV "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3D100E,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100 and Glu is substituted for Asp at amino acid position 106.

In a thirty-second 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50) with the proviso that any amino acid other than Ser

(denoted as "Z₃₁") is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the-amino-acid-sequence-of-Fig. 25 (SEQ_ID_NO: 50) with the proviso that any amino acid other than Ser (denoted as "Z₅₄") is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ_ID_NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106. In a preferred 6G4.2.5HV CDRs variant (referred to herein as "6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K,D106E"), H1 correspond to amino acids 26-35 of the amino acid sequence of Fig. 25 (SEQ_ID_NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 31, H2 corresponds to amino acids 50-66 of the amino acid sequence of Fig. 25 (SEQ_ID_NO: 50) with the proviso that Ala is substituted for Ser at amino acid position 54, and H3 corresponds to amino acids 99-111 of the amino acid sequence of Fig. 25 (SEQ_ID_NO: 50) with the proviso that Glu is substituted for Asp at amino acid position 100, Lys is substituted for Arg at amino acid position 102 and Glu is substituted for Asp at amino acid position 106.

As in the humanization of the light chain variable domain described above, a humanized heavy chain variable domain is constructed by substituting the CDRs of 6G4.2.5HV or the CDRs of a 6G4.2.5HV CDRs variant for the corresponding sequences in a human heavy chain variable domain. The humanized heavy chain variable domain comprising the CDRs of 6G4.2.5HV or the CDRs of a 6G4.2.5HV CDRs variant can also contain some FR residues that are substituted by residues from analogous sites in the murine 6G4.2.5 antibody heavy chain variable domain. The complete amino acid sequence of 6G4.2.5HV is set out as amino acids 1-122 of the amino acid sequence of Fig. 25 (SEQ ID NO: 50).

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies and antibody fragments is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework can be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993)).

It is also important that the antibodies and antibody fragments of the invention be humanized with retention of high affinity for human IL-8 and other favorable biological properties. To achieve this goal, according to a preferred method, the humanized antibodies and antibody fragments of the invention are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely

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role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and parental sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved.

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV are collectively referred to herein as "hu6G4.2.5LV".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35X₃₅ are collectively referred to herein as "hu 6G4.2.5LV/L1N35X₃₅".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35A are collectively referred to herein as "hu6G4.2.5LV/L1N35A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35E are collectively referred to herein as "hu6G4.2.5LV/L1N35E".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26X₂₆ are collectively referred to herein as "hu6G4.2.5LV/L1S26X₂₆".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26A are collectively referred to herein as "hu6G4.2.5LV/L1S26A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L3H98X₉₈ are collectively referred to herein as "hu6G4.2.5LV/L3H98X₉₈".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L3H98A are collectively referred to herein as "hu6G4.2.5LV/L3H98A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26X₂₆,N35X₃₅ are collectively referred to herein as "hu6G4.2.5LV/L1S26X₂₆,N35X₃₅".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26A,N35A are collectively referred to herein as "hu6G4.2.5LV/L1S26A,N35A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35X₃₅/L3H98X₉₈ are collectively referred to herein as

"hu6G4.2.5LV/L1N35X35/L3H98X98".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1N35A/L3H98A are collectively referred to herein as "hu6G4.2.5LV/L1N35A/L3H98A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of $6G4.2.5LV/L1S26X_{26}/L3H98X_{98}$ are collectively referred to herein as "hu $6G4.2.5LV/L1S26X_{26}/L3H98X_{98}$ ".

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Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26A/L3H98A are collectively referred to herein as

"hu6G4.2.5LV/L1S26A/L3H98A".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26X₂₆,N35X₃₅/L3H98X₉₈ are collectively referred to herein as "hu6G4.2.5LV/L1S26X₂₆,N35X₃₅/L3H98X₉₈".

Any and all humanized light chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5LV/L1S26A,N35A/L3H98A are collectively referred to herein as "hu6G4.2.5LV/L1S26A,N35A/L3H98A".

The humanized light chain variable domain amino acid sequences of hu6G4.2.5LV/L1N35 X_{35} , hu6G4.2.5LV/L1S26 X_{26} , hu6G4.2.5LV/L1S26 X_{26} /L3H98 X_{98} , hu6G4.2.5LV/L1S26 X_{26} ,N35 X_{35} , hu6G4.2.5LV/L1N35 X_{35} /L3H98 X_{98} , hu6G4.2.5LV/L1S26 X_{26} /L3H98 X_{98} , and hu6G4.2.5LV/L1S26 X_{26} /N35 X_{35} /L3H98 X_{98} are collectively referred to herein as "hu6G4.2.5LV/vL1-3X".

The humanized light chain variable domain amino acid sequences of hu6G4.2.5LV/L1N35A, hu6G4.2.5LV/L1S26A, hu6G4.2.5LV/L1S26A/L3H98A, hu6G4.2.5LV/L1S26A,N35A, hu6G4.2.5LV/L1N35A/L3H98A, hu6G4.2.5LV/L1S26A/L3H98A, hu6G4.2.5LV/L1S26A,N35A/L3H98A are collectively referred to herein as "hu6G4.2.5LV/vL1-3A".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV are collectively referred to herein as "hu6G4.2.5HV".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of $6G4.2.5HV/H1S31Z_{31}$ are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A are collectively referred to herein as "hu6G4.2.5HV/H1S31A".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄ are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A are collectively referred to herein as "hu6G4.2.5HV/H2S54A".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3R102K are collectively referred to herein as "hu6G4.2.5HV/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3D106E are collectively referred to herein as "hu6G4.2.5HV/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the

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CDRs of 6G4.2.5HV/H3D100E,R102K are collectively referred to herein as "hu6G4.2.5HV/H3D100E,R102K".

Any-and-all-humanized-heavy-chain-variable-domain-amino-acid-sequences-which-comprise-the CDRs of 6G4.2.5HV/H3R102K,D106E are collectively referred to herein as

5 "hu6G4.2.5HV/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of $6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}$ are collectively referred to herein as "hu $6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}$ ".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3R102K are collectively referred to herein as

20 "hu6G4.2.5HV/H1S31Z₃₁/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H3D100E,D106E".

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Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of $6G4.2.5HV/H2S54Z_{54}/H3R102K$ are collectively referred to herein as "hu $6G4.2.5HV/H2S54Z_{54}/H3R102K$ ".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3D106E are collectively referred to herein as

"hu6G4.2.5HV/H2S54Z₅₄/H3D106E".

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Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54Z₅₄/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of $6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}/H3D100E$ are collectively referred to herein as "hu $6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}/H3D100E$ ".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D106E are collectively referred to herein as

30 "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of $6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}/H3D100E,D106E$ are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H2S54A".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H3R102K are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H3D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H3D100E,R102K are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the

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CDRs of 6G4.2.5HV/H1S31A/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H3D100E,R102K,D106E".

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Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H2S54A/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3R102K are collectively referred to herein as "hu6G4.2.5HV/H2S54A/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54A/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54A/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3D100E,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54A/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H2S54A/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H2S54A/H3D100E,R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D100E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H2S54A/H3D100E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3R102K are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H2S54A/H3R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H2S54A/H3D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H2S54A/H3R102K,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D100E,D106E are collectively referred to herein as

"hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,D106E".

Any and all humanized heavy chain variable domain amino acid sequences which comprise the CDRs of 6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K,D106E".

The humanized heavy chain variable domain amino acid sequences of hu6G4.2.5HV/H1S31Z₃₁, hu6G4.2.5HV/H2S54Z₅₄, hu6G4.2.5HV/H3D100E, hu6G4.2.5HV/H3R102K, hu6G4.2.5HV/H3D106E, hu6G4.2.5HV/H3D100E,R102K, hu6G4.2.5HV/H3D100E,D106E, hu6G4.2.5HV/H3D100E,R102K,D106E, hu6G4.2.5HV/H3D100E,D106E, hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄, hu6G4.2.5HV/H1S31Z₃₁/H3D100E,

10 hu6G4.2.5HV/H1S31Z₃₁/H3R102K, hu6G4.2.5HV/H1S31Z₃₁/H3D106E,

 ${\tt hu6G4.2.5HV/H1S31Z_{31}/H3D100E,R102K,hu6G4.2.5HV/H1S31Z_{31}/H3R102K,D106E,hu6G4.2.5HV/H1S31Z_{31}/H3R102K,D106E,hu6G4.2.5HV/H1S31Z_{31}/H3R102K,D106E,hu6G4.2.5HV/H1S31Z_{31}/H3R102K,D106E,hu6G4.2.5HV/H1S31Z_{31}/H3R102K,hu6G4.2.5HV/H1S31Z_{31}/H3R$

hu6G4.2.5HV/H1S31Z₃₁/H3D100E,D106E, hu6G4.2.5HV/H1S31Z₃₁/H3D100E,R102K,D106E,

hu6G4.2.5HV/H2S54Z54/H3D100E, hu6G4.2.5HV/H2S54Z54/H3R102K,

 $hu6G4.2.5HV/H2S54Z_{54}/H3D106E, hu6G4.2.5HV/H2S54Z_{54}/H3R102K, D106E, hu6C4, hu6C4,$

15 54/H3D100E,D106E, hu6G4.2.5HV/H2S54Z54/H3D100E,R102K,D106E,

 $hu6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}/H3D100E, hu6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}/H3R102K, hu6G4.2.5HV/H1S31Z_{54}/H3R102K, hu6G4.2.5HV/H1S31Z_{54}/H3R102K$

hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D106E, hu6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄/H3D100E,R102K,

hu6G4.2.5HV/H1S31Z31/H2S54Z54/H3R102K,D106E,

 $hu6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}/H3D100E, D106E, and \ hu6G4.2.5HV/H1S31Z_{31}/H2S54Z_{54}/H3D100E, D106E, D10$

20 54/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/vH1-3Z".

The humanized heavy chain variable domain amino acid sequences of hu6G4.2.5HV/H1S31A, hu6G4.2.5HV/H2S54A, hu6G4.2.5HV/H3D100E, hu6G4.2.5HV/H3R102K, hu6G4.2.5HV/H3D106E, hu6G4.2.5HV/H3D100E,R102K, hu6G4.2.5HV/H3R102K,D106E, hu6G4.2.5HV/H3D100E,R102K,D106E,

25 hu6G4.2.5HV/H1S31A/H2S54A, hu6G4.2.5HV/H1S31A/H3D100E, hu6G4.2.5HV/H1S31A/H3R102K,

hu6G4.2.5HV/H1S31A/H3D106E, hu6G4.2.5HV/H1S31A/H3D100E,R102K,

hu6G4.2.5HV/H1S31A/H3R102K,D106E, hu6G4.2.5HV/H1S31A/H3D100E,D106E,

hu6G4.2.5HV/H1S31A/H3D100E,R102K,D106E, hu6G4.2.5HV/H2S54A/H3D100E,

hu6G4.2.5HV/H2S54A/H3R102K, hu6G4.2.5HV/H2S54A/H3D106E,

30 hu6G4.2.5HV/H2S54A/H3R102K,D106E, hu6G4.2.5HV/H2S54A/H3D100E,D106E,

hu6G4.2.5HV/H2S54A/H3D100E,R102K,D106E, hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,

hu6G4.2.5HV/H1S31A/H2S54A/H3R102K, hu6G4.2.5HV/H1S31A/H2S54A/H3D106E,

hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K, hu6G4.2.5HV/H1S31A/H2S54A/H3R102K,D106E, hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,D106E, and

hu6G4.2.5HV/H1S31A/H2S54A/H3D100E,R102K,D106E are collectively referred to herein as "hu6G4.2.5HV/vH1-3A".

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The invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3X. In another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3A. In yet another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35X35. In still another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35A. In a further embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35E.

The invention additionally provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3X, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z. In another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z. In yet another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/vL1-3A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5LV/vL1-3A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV/vH1-3A.

In a further embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35X₃₅, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z. In another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/N35X₃₅, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV/vH1-3A. In a preferred embodiment, the antibody or antibody fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35X₃₅ and further comprises a humanized heavy chain comprising the amino acid sequence of 6G4.2.5HV11.

In an additional embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z. In another embodiment, the invention provides a humanized antibody or antibody fragment that comprises a light chain variable domain comprising the hu6G4.2.5LV/N35A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV/vH1-3A. In still another embodiment, the humanized antibody or antibody

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fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35A, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV. In a further embodiment, the humanized antibody or antibody fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35E, and further comprises a heavy chain variable domain comprising the hu6G4.2.5HV. In a preferred embodiment, the antibody or antibody fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35A and further comprises a humanized heavy chain comprising the amino acid sequence of 6G4.2.5HV11. In another preferred embodiment, the antibody or antibody fragment comprises a light chain variable domain comprising the hu6G4.2.5LV/L1N35E and further comprises a humanized heavy chain comprising the amino acid sequence of 6G4.2.5HV11.

The invention encompasses a single chain antibody fragment comprising the hu6G4.2.5LV/vL1-3X, with or without any additional amino acid sequence. In one embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/vL1-3X without any associated heavy chain variable domain amino acid sequence, i.e. a single chain species that makes up one half of an Fv fragment. In another embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/vL1-3A without any associated heavy chain variable domain amino acid sequence. In still another embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/L1N35X₃₅ without any associated heavy chain variable domain amino acid sequence. In a preferred embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/L1N35A without any associated heavy chain variable domain amino acid sequence. In another preferred embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5LV/L1N35E without any associated heavy chain variable domain amino acid sequence.

In one embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/vL1-3X and the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/vL1-3X joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/vL1-3X joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In another embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/vL1-3A and the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/vL1-3A joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single

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chain antibody fragment is a species comprising the hu6G4.2.5LV/vL1-3A joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/vL1-3A and the hu6G4.2.5HV/vH1-3A are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/vL1-3A joined to the hu6G4.2.5HV/vH1-3A by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/vL1-3A joined to the hu6G4.2.5HV/vH1-3A by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In still another embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/L1N35X₃₅ and the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/L1N35X₃₅ joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35X₃₅ joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In a further embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/L1N35X₃₅ and the hu6G4.2.5HV/vH1-3A are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/L1N35X₃₅ joined to the hu6G4.2.5HV/vH1-3A by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35X₃₅ joined to the hu6G4.2.5HV/vH1-3A by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In an additional embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/L1N35A and the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species

comprising the hu6G4.2.5LV/L1N35A joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35A joined to the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

Also provided herein is a single chain antibody fragment wherein the hu6G4.2.5LV/L1N35E and the hu6G4.2.5HV are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/L1N35E joined to the hu6G4.2.5HV by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35E joined to the hu6G4.2.5HV by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In still another embodiment, the invention provides a single chain antibody fragment wherein the hu6G4.2.5LV/L1N35A and the hu6G4.2.5HV/vH1-3A are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5LV/L1N35A joined to the hu6G4.2.5HV/vH1-3A by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5LV/L1N35A joined to the hu6G4.2.5HV/vH1-3A by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3X and a second polypeptide chain comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3X and a second polypeptide chain comprises the hu6G4.2.5HV/vH1-3A and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3X and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

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In a further embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3A and a second polypeptide chain comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3A and a second polypeptide chain comprises the hu6G4.2.5HV/vH1-3A and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/vL1-3A and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

The invention also encompasses an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35X₃₅ and a second polypeptide chain comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35X₃₅ and a second polypeptide chain comprises the hu6G4.2.5HV/vH1-3A and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35X₃₅ and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

The invention further encompasses an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

The invention also encompasses an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprises the hu6G4.2.5HV and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprises the hu6G4.2.5HV/vH1-3A and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the invention provides an

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antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In another preferred embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprises the amino acid sequence of 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds.

In a preferred embodiment, any of the foregoing two-chain antibody fragments are selected from the group consisting of Fab, Fab'-SH, Fv, and F(ab') 2. In another preferred embodiment, the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab') 2, wherein the antibody fragment comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35X35 and a second polypeptide chain comprising the hu6G4.2.5HV. In yet another preferred embodiment, the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab')2, wherein the antibody fragment comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprising the hu6G4.2.5HV. In a further preferred embodiment, the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab')2, wherein the antibody fragment comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprising the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprising the hu6G4.2.5LV/L1N35A and a second polypeptide chain comprising the amino acid sequence of 6G4.2.5HV11. In an additional preferred embodiment, the antibody fragment is a F(ab')2 that comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprising the amino acid sequence of 6G4.2.5HV11. In an additional preferred embodiment, the antibody fragment is a F(ab')2 that comprises one polypeptide chain comprising the hu6G4.2.5LV/L1N35E and a second polypeptide chain comprising the amino acid sequence of 6G4.2.5HV11.

The invention also provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/vL1-3X and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including lgG, lgM, lgA, lgD, and lgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

The invention additionally provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/vL1-3X and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV/vH1-3A, wherein the light chain variable domain, and

optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention further provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35X₃₅ and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

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The invention additionally provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35X₃₅ and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV/vH1-3A, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention also encompasses an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35A and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

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The invention additionally provides an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35A and optionally further comprising a heavy chain variable domain containing the hu6G4.2.5HV/vH1-3A, wherein the light chain variable domain, and optionally the heavy chain variable domain, is (are) fused to an additional moiety, such as a immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including lgG, lgM, lgA, lgD, and lgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

The invention additionally encompasses an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35A and optionally further comprising a heavy chain containing the amino acid sequence of 6G4.2.5HV11, wherein the light chain variable domain, and optionally the heavy chain, is (are) fused to an additional moiety, such as immunoglobulin constant domain sequences. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.*

The invention further encompasses an antibody or antibody fragment comprising a light chain variable domain containing the hu6G4.2.5LV/L1N35E and optionally further comprising a heavy chain containing the amino acid sequence of 6G4.2.5HV11, wherein the light chain variable domain, and optionally the heavy chain, is (are) fused to an additional moiety, such as immunoglobulin constant domain sequences. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

In a preferred embodiment, the antibody or antibody fragment comprises a light chain variable domain containing the hu6G4.2.5LV/vL1-3X, and further comprises the hu6G4.2.5HV or hu6G4.2.5HV/vH1-3Z in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney et al., J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below.

In particular, the invention provides an antibody or antibody fragment comprising a light chain

comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35 (herein referred to as "6G4.2.5LV11N35X₃₅").

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In another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26 (herein referred to as "6G4.2.5LV11S26X₂₆").

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In yet another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than His (denoted as " X_{98} ") is substituted for His at amino acid position 98 (herein referred to as " $6G4.2.5LV11H98X_{98}$ ").

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In still another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Ser (denoted as "X₂₆") is substituted for Ser at amino acid position 26 and any amino acid other than Asn (denoted as "X₃₅") is substituted for Asn at amino acid position 35 (herein referred to as "6G4.2.5LV11S26X₂₆/N35X₃₅").

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In a further embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Asn (denoted as " X_{35} ") is substituted for Asn at amino acid position 35 and any amino acid other than His (denoted as " X_{98} ") is substituted for His at amino acid position 98 (herein referred to as " $6G4.2.5LV11N35X_{35}/H98X_{98}$ ").

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In an additional embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the provis that any amino acid other than Ser (denoted as " X_{26} ") is substituted for Ser at amino acid position 26 and any amino acid other than His (denoted as " X_{98} ") is substituted for His at amino acid position 98 (herein referred to as " $6G4.2.5LV11S26X_{26}/H98X_{98}$ ").

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The invention also encompasses an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that any amino acid other than Ser (denoted as " X_{26} ") is substituted for Ser at amino acid position 26, any amino acid other than Asn (denoted as " X_{35} ") is substituted for Asn at amino acid position 35 and any amino acid other than His (denoted as " X_{98} ") is substituted for His at amino acid position 98 (herein referred to as " G_{66} .2.5LV11S26 G_{26} N35 G_{98} ").

Additionally, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence (SEQ ID NO: 71) of Fig. 36 (herein referred to as "6G4.2.5LV11N35A").

Further provided herein is an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence (SEQ ID NO: 71) of Fig. 45 (herein referred to as "6G4.2.5LV11N35E").

In another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Ser at amino acid position 26 (herein referred to as "6G4.2.5LV11S26A").

In yet another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11H98A").

In still another embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Ser at amino acid position 26 and Ala is substituted for Asn at amino acid position 35 (herein referred to as "6G4.2.5LV11S26A/N35A").

In a further embodiment, the invention provides an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Ser at amino acid position 26 and Ala is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11S26A/H98A").

The invention also encompasses an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Asn at amino acid position 35 and Ala is substituted for His at amino acid position 98 (herein

referred to as "6G4.2.5LV11N35A/H98A").

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The invention further encompasses an antibody or antibody fragment comprising a light chain comprising the amino acid sequence of amino acids 1-219 of the variant humanized anti-IL-8 6G4.2.5v11 light chain polypeptide amino acid sequence of Fig. 31B (SEQ ID NO: 65) with the proviso that Ala is substituted for Ser at amino acid position 26, Ala is substituted for Asn at amino acid position 35, and Ala is substituted for His at amino acid position 98 (herein referred to as "6G4.2.5LV11S26A/N35A/H98A").

The invention provides a single chain antibody fragment comprising a variant light chain selected from the group consisting of 6G4.2.5LV11N35X₃₅, 6G4.2.5LV11S26X₂₆, 6G4.2.5LV11H98X₉₈, 6G4.2.5LV11S26X₂₆/ N35X₃₅, 6G4.2.5LV11N35X₃₅/ H98X₉₈, 6G4.2.5LV11S26X₂₆/H98X₉₈, and 6G4.2.5LV11S26X₂₆/ N35X₃₅/H98X₉₈, with or without any additional amino acid sequence. It will be understood that the group consisting of 6G4.2.5LV11N35X₃₅, 6G4.2.5LV11S26X₂₆, 6G4.2.5LV11H98X 98, 6G4.2.5LV11S26X₂₆/ N35X₃₅, 6G4.2.5LV11N35X₃₅/ H98X₉₈, 6G4.2.5LV11S26X₂₆/H98X₉₈, and 6G4.2.5LV11S26X₂₆/ N35X₃₅/H98X₉₈, is collectively referred to herein as the "group of 6G4.2.5LV11X variants", and that individual members of this group are generically referred to herein as a "6G4.2.5LV11X variant." In one embodiment, the invention provides a single chain antibody fragment comprising a 6G4.2.5LV11X variant without any associated heavy chain amino acid sequence, i.e. a single chain species that makes up one half of a Fab fragment. In a preferred embodiment, the invention provides a 6G4.2.5LV11N35X₃₅ variant without any associated heavy chain amino acid sequence.

The invention encompasses a single chain antibody fragment comprising a variant light chain selected from the group consisting of 6G4.2.5LV11N35A, 6G4.2.5LV11S26A, 6G4.2.5LV11H98A, 6G4.2.5LV11S26A/H98A, H98A, 6G4.2.5LV11N35A/ 6G4.2.5LV11S26A/ N35A, 6G4.2.5LV11S26A/ N35A/H98A, with or without any additional amino acid sequence. It will be understood that the group consisting of 6G4.2.5LV11N35A, 6G4.2.5LV11S26A, 6G4.2.5LV11H98A, 6G4.2.5LV11S26A/H98A, H98A, 6G4.2.5LV11N35A/ N35A, 6G4.2.5LV11S26A/ 6G4.2.5LV11S26A/ N35A/H98A is collectively referred to herein as the "group of 6G4.2.5LV11A variants", and that individual members of this group are generically referred to herein as a "6G4.2.5LV11A In one embodiment, the invention provides a single chain antibody fragment comprising a 6G4.2.5LV11A variant without any associated heavy chain amino acid sequence, i.e. a single chain species that makes up one half of a Fab fragment. In a preferred embodiment, the invention provides the 6G4.2.5LV11N35A without any associated heavy chain amino acid sequence.

Further provided herein are an antibody or antibody fragment comprising a light chain comprising a 6G4.2.5LV11X variant, and further comprising a heavy chain comprising the 6G4.2.5HV11. In a preferred embodiment, the invention provides an antibody or antibody fragment comprising a 6G4.2.5LV11N35X35 variant and further comprising the 6G4.2.5HV11. In a preferred embodiment, the

invention provides an antibody or antibody fragment comprising the 6G4.2.5LV11N35A and further comprising the 6G4.2.5HV11. In another preferred embodiment, the invention provides an antibody or antibody fragment comprising the 6G4.2.5LV11N35E and further comprising the 6G4.2.5HV11.

In one embodiment, the invention provides a single chain antibody fragment wherein a 6G4.2.5LV11X variant and the 6G4.2.5HV11 are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment comprises a 6G4.2.5LV11X variant joined to the 6G4.2.5HV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising a 6G4.2.5LV11X variant joined to the 6G4.2.5HV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In still another embodiment, the invention provides a single chain antibody fragment wherein a 6G4.2.5LV11N35X35 variant and the 6G4.2.5HV11 are contained in a single chain polypeptide species. In

a preferred embodiment, the single chain antibody fragment comprises a 6G4.2.5LV11N35X₃₅ variant joined to the 6G4.2.5HV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising a 6G4.2.5LV11N35X₃₅ variant joined to the 6G4.2.5HV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In a further embodiment, the invention provides a single chain antibody fragment wherein the 6G4.2.5LV11N35A and the 6G4.2.5HV11 are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment comprises the 6G4.2.5LV11N35A joined to the 6G4.2.5HV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising the 6G4.2.5LV11N35A joined to the 6G4.2.5HV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another

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In an additional embodiment, the invention provides a single chain antibody fragment wherein the 6G4.2.5LV11N35E and the 6G4.2.5HV11 are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment comprises the 6G4.2.5LV11N35E joined to the 6G4.2.5HV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In another embodiment, the single chain antibody fragment is a species comprising the 6G4.2.5LV11N35E

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joined to the 6G4.2.5HV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises a 6G4.2.5LV11X variant and a second polypeptide chain comprises the 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In still another embodiment, the invention provides an antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises a 6G4.2.5LV11N35X35 variant and a second polypeptide chain comprises the 6G4.2.5HV11 and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, any of the foregoing two-chain antibody fragments is selected from the group consisting of Fab, Fab', Fab'-SH, and F(ab')2. In still another preferred embodiment, the two-chain antibody fragment is a F(ab')₂ wherein one polypeptide chain comprises the 6G4.2.5LV11N35A and the second polypeptide chain comprises the 6G4.2.5HV11. In a further preferred embodiment, the antibody fragment is a Fab, Fab', Fab'-SH, or F(ab')2 wherein one polypeptide chain comprises the 6G4.2.5LV11N35E and the second polypeptide chain comprises the 6G4.2.5HV11. A particularly preferred embodiment, the antibody fragment is the 6G4V11N35A F(ab')2 GCN4 leucine zipper species described in the Examples below. In another particularly preferred embodiment, the antibody fragment is the 6G4V11N35E F(ab')2 GCN4 leucine zipper species described in the Examples below. In yet another particularly preferred embodiment, the antibody fragment is the 6G4V11N35E Fab described in the Examples below.

The invention also provides an antibody or antibody fragment comprising a light chain containing a 6G4.2.5LV11X variant and optionally further comprising a heavy chain containing the 6G4.2.5HV11, wherein the light chain, and optionally the heavy chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/ r light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

The invention additionally provides an antibody or antibody fragment comprising a light chain containing a 6G4.2.5LV11N35X₃₅ variant and optionally further comprising a heavy chain containing the 6G4.2.5HV11, wherein the light chain, and optionally the heavy chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full r partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose,

including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

The invention further provides an antibody or antibody fragment comprising a light chain containing the 6G4.2.5LV11N35A and optionally further comprising a heavy chain containing the 6G4.2.5HV11, wherein the light chain, and optionally the heavy chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

The invention further provides an antibody or antibody fragment comprising a light chain containing the 6G4.2.5LV11N35E and optionally further comprising a heavy chain containing the 6G4.2.5HV11, wherein the light chain, and optionally the heavy chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

In a preferred embodiment, the antibody or antibody fragment comprises a light chain containing a 6G4.2.5LV11X variant, and further comprises the 6G4.2.5HV11 in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney et al., J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below. In another preferred embodiment, the antibody or antibody fragment comprises a light chain containing the 6G4.2.5LV11N35A, and further comprises a heavy chain containing the 6G4.2.5HV11 fused to the GCN4 leucine zipper. In yet another preferred embodiment, the antibody or antibody fragment comprises a light chain containing the 6G4.2.5LV11N35E, and further comprises a heavy chain containing the 6G4.2.5HV11 fused to the GCN4 leucine zipper.

B. <u>6G4.2.5HV VARIANTS</u>

The invention provides humanized antibodies and antibody fragments comprising the CDRs of a 6G4.2.5HV CDR variant. The use of a 6G4.2.5HV CDRs variant in the humanized antib dies and antibody fragments of the invention confer the advantages of higher affinity for human IL-8 and/or improved recombinant manufacturing economy.

A heavy chain variable domain comprising the CDRs of a 6G4.2.5HV CDRs variant can be

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humanized in conjunction with a light chain comprising the CDRs of 6G4.2.5LV or the CDRs of a 6G4.2.5LV CDRs variant, essentially as described in Section (II)(2)(A) above. In one embodiment, the invention provides a humanized antibody or antibody fragment comprising a 6G4.2.5HV CDRs variant selected from the group consisting of 6G4.2.5HV/H1S31Z₃₁, 6G4.2.5HV/H2S54Z₅₄, and 6G4.2.5HV/H1S31Z₃₁/H2S54Z₅₄. In addition, the invention provides a humanized antibody or antibody fragment comprising a 6G4.2.5HV CDRs variant selected from the group consisting of 6G4.2.5HV/H1S31A, 6G4.2.5HV/H2S54A, and 6G4.2.5HV/H1S31A/H2S54A. In particular, the 6G4.2.5HV CDRs variants can be used to construct a humanized antibody or antibody comprising the hu6G4.2.5HV/vH1-3Z as described in Section (II)(2)(A) above.

The invention additionally provides a humanized antibody or antibody fragment that comprises a heavy chain variable domain comprising the hu6G4.2.5HV/vH1-3Z, and further comprises a light chain variable domain comprising the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X.

The invention further encompasses a single chain humanized antibody fragment comprising the hu6G4.2.5HV/vH1-3Z, with or without any additional amino acid sequence. In one embodiment, the invention provides a single chain antibody fragment comprising the hu6G4.2.5HV/vH1-3Z without any associated heavy chain variable domain amino acid sequence, i.e. a single chain species that makes up one half of an Fv fragment.

In one embodiment, the invention provides a single chain humanized antibody fragment wherein the hu6G4.2.5HV/vH1-3Z and the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X are contained in a single chain polypeptide species. In a preferred embodiment, the single chain antibody fragment is a scFv species comprising the hu6G4.2.5HV/vH1-3Z joined to the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X by means of a flexible peptide linker sequence, wherein the heavy chain and light chain variable domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fv species. In another embodiment, the single chain antibody fragment is a species comprising the hu6G4.2.5HV/vH1-3Z joined to the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X by a linker that is too short to permit intramolecular pairing of the two variable domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides a humanized antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises the hu6G4.2.5HV/vH1-3Z and a second polypeptide chain comprises the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the foregoing two-chain antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, and F(ab') 2.

The invention also provides a humanized antibody or antibody fragment comprising a heavy chain variable domain containing the hu6G4.2.5HV/vH1-3Z and optionally further comprising a light chain variable domain containing the hu6G4.2.5LV or hu6G4.2.5LV/vL1-3X, wherein the heavy chain variable

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domain, and optionally the light chain variable domain, is (are) fused to an additional moiety, such as an immunoglobulin constant domain. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat et al.

In a preferred embodiment, the humanized antibody or antibody fragment comprises the hu6G4.2.5HV/vH1-3Z in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney et al., J. Immunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below.

In addition, the invention provides a humanized antibody or antibody fragment comprising a heavy chain comprising the amino acid sequence of amino acids 1-230 of the 6G4.2.5HV11 polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75) with the proviso that Ala is substituted for Ser at amino acid position 31 (hereinafter referred to as "6G4.2.5HV11S31A").

In another embodiment, the invention provides a humanized antibody or antibody fragment comprising a heavy chain comprising the amino acid sequence of amino acids 1-230 of the 6G4.2.5HV11 polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75) with the proviso that Ala is substituted for Ser at amino acid position 54 (hereinafter referred to as "6G4.2.5HV11S54A").

In yet another embodiment, the invention provides a humanized antibody or antibody fragment comprising a heavy chain comprising the amino acid sequence of amino acids 1-230 of the 6G4.2.5HV11 polypeptide amino acid sequence of Figs. 37A-37B (SEQ ID NO: 75) with the proviso that Ala is substituted for Ser at amino acid position 31 and Ala is substituted for Ser at amino acid position 54 (hereinafter referred to as "6G4.2.5HV11S31A/S54A").

Further provided herein is a humanized antibody or antibody fragment that comprises any of the light and heavy chain combinations listed in Tables 1 and 2 below.

Table 1

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30	Heavy Chain	Light Chain
	6G4.2.5HV11S31A	6G4.2.5LV11
	6G4.2.5HV11S31A	6G4.2.5LV11N35A
	6G4.2.5HV11S31A	6G4.2.5LV11S26A
	6G4.2.5HV11S31A	6G4.2.5LV11H98A
35	6G4.2.5HV11S31A	6G4.2.5LV11S26A/N35A
-	6G4.2.5HV11S31A	6G4.2.5LV11S26A/H98A
	6G4.2.5HV11S31A	6G4.2.5LV11N35A/H98A
	6G4.2.5HV11S31A	6G4.2.5LV11S26A/N35A/H98A
	6G4.2.5HV11S54A	6G4.2.5LV11
40	6G4.2.5HV11S54A	6G4.2.5LV11N35A
	6G4.2.5HV11S54A	6G4.2.5LV11S26A
	6G4.2.5HV11S54A	6G4.2.5LV11H98A

Table 2

lable 2	
y Chain	Light Chain
2.5HV11S54A	6G4.2.5LV11S26A/N35A
2.5HV11S54A	6G4.2.5LV11S26A/H98A
2.5HV11S54A	6G4.2.5LV11N35A/H98A
2.5HV11S54A	6G4.2.5LV11S26A/N35A/H98A
2.5HV11S31A/S54A	6G4.2.5LV11
2.5HV11S31A/S54A	6G4.2.5LV11N35A 6G4.2.5LV11S26A
2.5HV11S31A/S54A 2.5HV11S31A/S54A	6G4.2.5LV11H98A
2.5HV11S31A/S54A	G4.2.5LV11S26A/N35A
2.5HV11S31A/S54A	6G4.2.5LV11S26A/H98A
2.5HV11S31A/S54A	6G4.2.5LV11N35A/H98A
2.5HV11S31A/S54A	6G4.2.5LV11S26A/N35A/H98A
2.5HV11S31A	6G4.2.5LV11
2.5HV11S31A	6G4.2.5LV11N35X ₃₅
2.5HV11S31A	6G4.2.5LV11S26X ₂₆
2.5HV11S31A	6G4.2.5LV11H98X ₉₈
2.5HV11S31A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅
2.5HV11S31A	6G4.2.5LV11S26X ₂₆ /H98X ₉₈
2.5HV11S31A	6G4.2.5LV11N35X ₃₅ /H98X ₉₈
2.5HV11S31A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅ /H98X ₉₈
2.5HV11S54A	6G4.2.5LV11
2.5HV11S54A	6G4.2.5LV11N35X ₃₅
2.5HV11S54A	6G4.2.5LV11S26X ₂₆
2.5HV11S54A	6G4.2.5LV11H98X ₉₈
2.5HV11S54A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅
2.5HV11S54A	6G4.2.5LV11S26X ₂₆ /H98X ₉₈
2.5HV11S54A	6G4.2.5LV11N35X ₃₅ /H98X ₉₈
.2.5HV11S54A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅ /H98X ₉₈
.2.5HV11S31A/S54A	6G4.2.5LV11
.2.5HV11S31A/S54A	6G4.2.5LV11N35X ₃₅
.2.5HV11S31A/S54A	6G4.2.5LV11S26X ₂₆
.2.5HV11S31A/S54A	6G4.2.5LV11H98X ₉₈
.2.5HV11S31A/S54A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅
.2.5HV11S31A/S54A	6G4.2.5LV11S26X ₂₆ /H98X ₉₈
.2.5HV11S31A/S54A	6G4.2.5LV11N35X ₃₅ /H98X ₉₈
.2.5HV11S31A/S54A	6G4.2.5LV11S26X ₂₆ /N35X ₃₅ /H98X ₉₈

The invention encompasses a single chain humanized antibody fragment comprising a variant heavy chain selected from the group consisting of 6G4.2.5HV11S31A, 6G4.2.5HV11S54A, and 6G4.2.5HV11S31A/S54A, with or without any additional amino acid sequence. It will be understood that the group consisting of 6G4.2.5HV11S31A, 6G4.2.5HV11S54A, and 6G4.2.5HV11S31A/S54A is collectively referred to herein as the "group of 6G4.2.5HV11A variants", and that individual members of

this group are generically referred to herein as a "6G4.2.5HV11A variant." In one embodiment, the invention provides a single chain humanized antibody fragment comprising a 6G4.2.5HV11A variant without any associated light chain amino acid sequence, i.e. a single chain species that makes up one half of a Fab fragment.

Further provided herein are a humanized antibody or antibody fragment comprising a heavy chain comprising a 6G4.2.5HV11A variant, and further comprising a light chain comprising a 6G4.2.5LV11A variant or a 6G4.2.5LV11X variant. In another embodiment, the humanized antibody or antibody fragment comprises any combination of light and heavy chains listed in Tables 1 and 2 above. In one embodiment, the invention provides a humanized antibody or antibody fragment comprising a 6G4.2.5HV11A variant and further comprising the 6G4.2.5LV11N35X35. In a preferred embodiment, the invention provides a humanized antibody or antibody fragment comprising a 6G4.2.5HV11A variant and further comprising the 6G4.2.5LV11N35A.

In yet another embodiment, the invention provides a single chain humanized antibody fragment wherein a 6G4.2.5HV11A variant and the 6G4.2.5LV11 are contained in a single chain polypeptide species. In another embodiment, the invention provides a single chain humanized antibody fragment wherein any pair of light and heavy chains listed in Tables 1 and 2 above is contained in a single chain polypeptide species. In yet another embodiment, the invention provides a single chain humanized antibody fragment wherein a 6G4.2.5HV11A variant and a 6G4.2.5LV11X variant are contained in a single chain polypeptide species. In still another embodiment, the invention provides a single chain humanized antibody fragment wherein a 6G4.2.5HV11A variant and a 6G4.2.5LV11N35X₃₅ variant are contained in a single chain polypeptide species. In an additional embodiment, the invention provides a single chain humanized antibody fragment wherein a 6G4.2.5HV11A variant and the 6G4.2.5LV11N35A variant are contained in a single chain polypeptide species.

In a preferred embodiment, the single chain humanized antibody fragment comprises a 6G4.2.5HV11A variant joined to a 6G4.2.5LV11X variant, 6G4.2.5LV11N35X₃₅ variant, 6G4.2.5LV11N35A variant, or 6G4.2.5LV11 by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In a further embodiment, the single chain humanized antibody fragment is a species comprising a 6G4.2.5HV11A variant joined to a 6G4.2.5LV11X variant, 6G4.2.5LV11N35X₃₅ variant, 6G4.2.5LV11N35A variant, or 6G4.2.5LV11 by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In still another embodiment, the single chain humanized antibody fragment comprises any pair of light and heavy chains listed in Tables 1 and 2 above joined by means of a flexible peptide linker sequence, wherein the heavy chain and light chain domains can associate in a "dimeric" structure analogous to that formed in a two-chain Fab species. In an additional embodiment, the single chain humanized antibody

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fragment comprises any pair of light and heavy chains listed in Tables 1 and 2 above joined by a linker that is too short to permit intramolecular pairing of complementary domains, i.e. a single chain polypeptide monomer that forms a diabody upon dimerization with another monomer.

In yet another embodiment, the invention provides a humanized antibody fragment comprising a plurality of polypeptide chains, wherein one polypeptide chain comprises a 6G4.2.5HV11A variant and a second polypeptide chain comprises a 6G4.2.5LV11X variant, 6G4.2.5LV11N35X₃₅ variant, 6G4.2.5LV11N35A variant, or 6G4.2.5LV11, and the two polypeptide chains are covalently linked by one or more interchain disulfide bonds. In a preferred embodiment, the foregoing two-chain antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, and F(ab')₂.

In an additional embodiment, the invention provides a two-chain humanized antibody fragment comprising any pair of heavy and light chains listed in Tables 1 and 2 above, wherein each chain is contained on a separate molecule. In another embodiment, the two-chain antibody fragment comprising any pair of heavy and light chains listed in Tables 1 and 2 above is selected from the group consisting of Fab, Fab'-SH, and F(ab') 2. In a preferred embodiment, the two-chain humanized antibody fragment is a F(ab') 2 comprising any pair of heavy and light chains listed in Tables 1 and 2 above. In another preferred embodiment, the two-chain humanized antibody fragment is a F(ab') 2 wherein one polypeptide chain comprises a 6G4.2.5HV11A variant and the second polypeptide chain comprises the 6G4.2.5LV11N35A.

The invention also provides a humanized antibody or antibody fragment comprising a heavy chain containing a 6G4.2.5HV11A variant and optionally further comprising a light chain containing a 6G4.2.5LV11X variant, 6G4.2.5LV11N35X₃₅ variant, 6G4.2.5LV11N35A, or 6G4.2.5HV11, wherein the heavy chain, and optionally the light chain, is (are) fused to an additional moiety, such as additional immunoglobulin constant domain sequence. Constant domain sequence can be added to the heavy chain and/or light chain sequence(s) to form species with full or partial length heavy and/or light chain(s). It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species. Preferably, the constant domain sequence is human in origin. Suitable human constant domain sequences can be obtained from Kabat *et al.* (supra).

In a preferred embodiment, the humanized antibody or antibody fragment comprises a 6G4.2.5HV11A variant in a heavy chain that is fused to or contains a leucine zipper sequence. The leucine zipper can increase the affinity or production efficiency of the antibody or antibody fragment of interest. Suitable leucine zipper sequences include the jun and fos leucine zippers taught by Kostelney et al., J. lmmunol., 148: 1547-1553 (1992) and the GCN4 leucine zipper described in the Examples below.

C. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding

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specificities is for IL-8, the other one is for any other antigen. For example, bispecific antibodies specifically binding a IL-8 and neurotrophic factor, or two different types of IL-8 polypeptides are within the scope of the present-invention.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published 13 May 1993, and in Traunecker et al., EMBO J. 10:3655 (1991).

According to a different and more preferred approach, antibody-variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant-domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1), containing the site necessary for light-chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the maximum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the production of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. For further details of generating bispecific antibodies, see, for example, Suresh et al., Methods in Enzymology 121:210 (1986).

According to another approach, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as

homodimers.

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Bispecific antib dies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/00373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab') 2 fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from E. coli, which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med., 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab') 2 molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol., 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., J. Immunol., 152:5368 (1994).

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Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al. J. Immunol. 147: 60 (1991).

4. Production of Humanized Anti-IL-8 6G4.2.5 Monoclonal Antibody, Antibody Fragments, and Variants

The antibodies and antibody fragments of the invention can be produced using any convenient antibody manufacturing process known in the art. Typically, the antibody or antibody fragment is made using recombinant expression systems. A multiple polypeptide chain antibody or antibody fragment species can be made in a single host cell expression system wherein the host cell produces each chain of the antibody or antibody fragment and assembles the polypeptide chains into a multimeric structure to form the antibody or antibody fragment in vivo, followed by recovery of the antibody or antibody fragment from the host cell. For example, suitable recombinant expression systems for the production of complete antibody or antibody fragment are described in Lucas et al., Nucleic Acids Res., 24: 1774-1779 (1996). Alternatively, the separate polypeptide chains of the desired antibody or antibody fragment can be made in separate expression host cells, separately recovered from the respective host cells, and then mixed in vitro under conditions permitting the formation of the multi-subunit antibody or antibody fragment of interest. For example, U.S. Pat. No. 4,816,567 to Cabilly et al. and Carter et al., Bio/Technology, 10: 163-167 (1992) provide methods for recombinant production of antibody heavy and light chains in separate expression hosts followed by assembly of antibody from separate heavy and light chains in vitro.

The following discussion of recombinant expression methods applies equally to the production of single chain antibody polypeptide species and multi-subunit antibody and antibody fragment species. All recombinant procedures for the production of antibody or antibody fragment provided below shall be understood to describe: (1) manufacture of single chain antibody species as the desired end-product; (2) manufacture of multi-subunit antibody or antibody fragment species by production of all subunits in a single host cell, subunit assembly in the host cell, optionally followed by host cell secretion of the multi-subunit end-product into the culture medium, and recovery of the multi-subunit end-product from the host cell and/or culture medium; and (3) manufacture of multi-subunit antibody or antibody fragment by production of subunits in separate host cells (optionally followed by host cell secretion of subunits into the culture medium), recovery of subunits from the respective host cells and/or culture media, followed by in vitro subunit assembly to form the multi-subunit end-product. In the case of a multi-subunit antibody or antibody fragment produced in a single host cell, it will be appreciated that production of the various subunits can be effected by expression of multiple polypeptide-encoding nucleic acid sequences carried on a single vector or by expression of polypeptide-encoding nucleic acid sequences carried on multiple vectors contained in the host cell.

A. Construction of DNA Encoding Humanized 6G4.2.5 Monoclonal Antibodies, Antibody Fragments, and Variants

Following the selection of the humanized antibody or antibody fragment of the invention according to the methods described above, the practitioner can use the genetic code to design DNAs

encoding the desired antibody or antibody fragment. In one embodiment, codons preferred by the expression host cell are used in the design of a DNA encoding the antibody or antibody fragment of interest. DNA encoding the desired antibody or antibody fragment can be prepared by a variety of methods known in the art. These methods include, but are not limited to, chemical synthesis by any of the methods described in Engels et al., Agnew. Chem. Int. Ed. Engl., 28: 716-734 (1989), the entire disclosure of which is incorporated herein by reference, such as the triester, phosphite, phosphoramidite and H-phosphonate methods.

A variation on the above procedures contemplates the use of gene fusions, wherein the gene(s) encoding the antibody or antibody fragment is associated, in the vector, with a gene encoding another protein or a fragment of another protein. This results in the antibody or antibody fragment being produced by the host cell as a fusion with another protein. The "other" protein is often a protein or peptide which can be secreted by the cell, making it possible to isolate and purify the desired protein from the culture medium and eliminating the necessity of destroying the host cells which arises when the desired protein remains inside the cell. Alternatively, the fusion protein can be expressed intracellularly. It is advantageous to use fusion proteins that are highly expressed.

The use of gene fusions, though not essential, can facilitate the expression of heterologous proteins in *E. coli* as well as the subsequent purification of those gene products (Harris, T. J. R. in *Genetic Engineering*, Williamson, R., Ed., Academic, London, Vol. 4, p. 127(1983); Uhlen, M. & Moks, T., *Methods Enzymol.* 185:129-143 (1990)). Protein A fusions are often used because the binding of protein A, or more specifically the Z domain of protein A, to IgG provides an "affinity handle" for the purification of the fused protein (Nilsson, B. & Abrahmsen, L. *Methods Enzymol.* 185:144-161 (1990)). It has also been shown that many heterologous proteins are degraded when expressed directly in *E. coli*, but are stable when expressed as fusion proteins (Marston, F. A. O., *Biochem J.* 240: 1 (1986)).

Fusion proteins can be cleaved using chemicals, such as cyanogen bromide, which cleaves at a methionine, or hydroxylamine, which cleaves between an Asn and Gly. Using standard recombinant DNA methodology, the nucleotide base pairs encoding these amino acids may be inserted just prior to the 5' end of the antibody or antibody fragment gene(s).

Alternatively, one can employ proteolytic cleavage of fusion proteins, which has been recently reviewed (Carter, P. (1990) in *Protein Purification: From Molecular Mechanisms to Large-Scale Processes*. Ladisch, M. R., Willson, R. C., Painton, C. C., and Builder, S. E., eds., American Chemical Society Symposium Series No. 427, Ch 13, 181-193).

Proteases such Factor Xa, thrombin, subtilisin and mutants thereof, have been successfully used to cleave fusion proteins. Typically, a peptide linker that is amenable to cleavage by the protease used is inserted between the "other" protein (e.g., the Z domain of protein A) and the protein of interest, such as humanized anti-IL-8 antibody or antibody fragment. Using recombinant DNA methodology, the nucleotide base pairs encoding the linker are inserted between the genes or gene fragments coding for the other proteins. Proteolytic cleavage of the partially purified fusion protein containing the correct linker can then

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be carried out on either the native fusion protein, or the reduced or denatured fusion protein.

Various techniques are also available which may now be employed to produce variant humanized antibodies or antibody fragments, which encodes for additions, deletions, or changes in amino acid sequence of the resultant protein(s) relative to the parent humanized antibody or antibody fragment.

By way of illustration, with expression vectors encoding humanized antibody or antibody fragment in hand, site specific mutagenesis (Kunkel et al., Methods Enzymol. 204:125-139 (1991); Carter, P., et al., Nucl. Acids. Res. 13:4331 (1986); Zoller, M. J. et al., Nucl. Acids Res. 10:6487 (1982)), cassette mutagenesis (Wells, J. A., et al., Gene 34:315 (1985)), restriction selection mutagenesis (Wells, J. A., et al., Philos. Trans, R. Soc. London SerA 317, 415 (1986)) or other known techniques may be performed on the antibody or antibody fragment DNA. The variant DNA can then be used in place of the parent DNA by insertion into the aforementioned expression vectors. Growth of host bacteria containing the expression vectors with the mutant DNA allows the production of variant humanized antibodies or antibody fragments, which can be isolated as described herein.

B. Insertion of DNA into a Cloning Vehicle

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The DNA encoding the antibody or antibody fragment is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available, and selection of the appropriate vector will depend on (1) whether it is to be used for DNA amplification or for DNA expression, (2) the size of the DNA to be inserted into the vector, and (3) the host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the host cell for which it is compatible. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(i) Signal Sequence Component

In general, a signal sequence may be a component of the vector, or it may be a part of the antibody or antibody fragment DNA that is inserted into the vector. Preferably, a heterologous signal sequence selected and fused to the antibody or antibody fragment DNA such that the signal sequence in the corresponding fusion protein is recognized, transported and processed (*i.e.*, cleaved by a signal peptidase) in the host cell's protein secretion system. In the case of prokaryotic host cells, the signal sequence is selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. In a preferred embodiment, the STII signal sequence is used as described in the Examples below. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, α factor leader (including Saccharomyces and Kluyveromyces α-factor leaders), or acid phosphatase leader, the C. albicans glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

(ii) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables

the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is ne that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2µ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, i.e. they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is homologous to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of the antibody or antibody fragment DNA.

(iii) Selection Gene Component

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Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g. ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene express a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin (Southern et al., J. Molec. Appl. Genet., 1: 327 (1982)), mycophenolic acid (Mulligan et al., Science, 209: 1422 (1980)) or hygromycin (Sugden et al., Mol. Cell. Biol., 5: 410-413 (1985)). The three examples given above employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug (G418 or neomycin (geneticin), xgpt (mycophenolic acid), and hygromycin, respectively.)

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody or antibody fragment nucleic acid, such as dihydrofolate reductase (DHFR) or thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of

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both the selection gene and the DNA that encodes the antibody or antibody fragment. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of the antibody or antibody fragment are synthesized from the amplified DNA.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 (1980). The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding the antibody or antibody fragment. This amplification technique can be used with any otherwise suitable host, e.g., ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060). Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the antibody or antibody fragment, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3' phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7. Stinchcomb *et al.*, Nature, 282: 39 (1979); Kingsman *et al.*, Gene, 7: 141 (1979); or Tschemper *et al.*, Gene, 10: 157 (1980). The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, Genetics, 85: 12 (1977). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

(iv) Promoter Component

Expression vectors usually contain a promoter that is recognized by the host organism and is operably linked to the antibody or antibody fragment nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of a particular nucleic acid sequence, such as the antibody or antibody fragment encoding sequence, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g. the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known.

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter

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systems (Chang et al., Nature, 275: 615 (1978); and Goeddel et al., Nature, 281: 544 (1979)), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, Nucleic Acids Res., 8: 4057 (1980) and EP 36,776) and hybrid promoters such as the tac promoter (deBoer et al., Proc. Natl. Acad. Sci. USA, 80: 21-25 (1983)). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker to operably ligate them to DNA encoding the antibody or antibody fragment (Siebenlist et al., Cell, 20: 269 (1980)) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also generally will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the antibody or antibody fragment.

Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman et al., J. Biol. Chem., 255: 2073 (1980)) or other glycolytic enzymes (Hess et al., J. Adv. Enzyme Reg., 7: 149 (1968); and Holland, Biochemistry, 17: 4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman et al., EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into mammalian expression vectors.

Vector driven transcription of antibody or antibody fragment encoding DNA in mammalian host cells can be controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers et al., Nature, 273: 113 (1978); Mulligan and Berg, Science, 209: 1422-1427 (1980); Pavlakis et al., Proc. Natl. Acad. Sci. USA, 78: 7398-7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway et al., Gene, 18: 355-360 (1982). A system for expressing DNA

in mammalian h sts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray et al., Nature, 295: 503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells, Reyes et al., Nature, 297: 598-601 (1982) on expression of human -interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79: 5166-5170 (1982) on expression of the human interferon 1 gene in cultured mouse and rabbit cells, and Gorman et al., Proc. Natl. Acad. Sci. USA, 79: 6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

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(v) Enhancer Element Component

Transcription of a DNA encoding antibody or antibody fragment by higher eukaryotic host cells is often increased by inserting an enhancer sequence into the vector. Enhancers are cisacting elements of DNA, usually about from 10-300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent having been found 5' (Laimins et al., Proc. Natl. Acad. Sci. USA, 78: 993 (1981)) and 3' (Lusky et al., Mol. Cell Bio., 3: 1108 (1983)) to the transcription unit, within an intron (Banerji et al., Cell., 33: 729 (1983)) as well as within the coding sequence itself (Osborne et al., Mol. Cell Bio., 4: 1293 (1984)). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, -fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297: 17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody or antibody fragment DNA, but is preferably located at a site 5' from the promoter.

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(vi) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) can also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3' untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the antibody or antibody fragment. The 3' untranslated regions also include transcription termination sites.

Suitable vectors containing one or more of the above listed components and the desired coding and control sequences are constructed by standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform E. coli K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or

tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing et al., Nucleic Acids Res., 9: 309 (1981) or by the method of Maxam et al., Methods in Enzymology, 65: 499 (1980).

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding the antibody or antibody fragment. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high I vels of a desired polypeptide encoded by the expression vector.

Other methods, vectors, and host cells suitable for adaptation to the synthesis of the antibody or antibody fragment in recombinant vertebrate cell culture are described in Gething et al., Nature, 293: 620-625 (1981); Mantei et al., Nature, 281: 40-46 (1979); Levinson et al., EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of the IgE peptide antagonist is pRK5 (EP pub. no. 307,247) or pSVI6B (PCT pub. no. WO 91/08291 published 13 June 1991).

C. Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, for example, E. coli, Bacilli such as B. subtilis, Pseudomonas species such as P. aeruginosa, Salmonella typhimurium, or Serratia marcescens. One preferred E. coli cloning host is E. coli 294 (ATCC 31,446), although other strains such as E. coli B, E. coli 1776 (ATCC 31,537), and E. coli W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Preferably the host cell should secrete minimal amounts of proteolytic enzymes. In a preferred embodiment, the E. coli strain 49D6 is used as the expression host as described in the Examples below. Review articles describing the recombinant production of antibodies in bacterial host cells include Skerra et al., Curr. Opinion in Immunol., 5: 256 (1993) and Pluckthun, Immunol. Revs., 130: 151 (1992).

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing antibody or antibody fragment DNA. Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as S. pombe (Beach and Nurse, Nature, 290: 140 (1981)), Kluyveromyces lactis (Louvencourt et al., J. Bacteriol., 737 (1983)), yarrowia (EP 402,226), Pichia pastoris (EP 183,070), Trichoderma reesia (EP 244,234), Neurospora crassa (Case et al., Proc. Natl. Acad. Sci. USA, 76: 5259-5263 (1979)), and Aspergillus hosts such as A. nidulans (Ballance et al., Biochem. Biophys. Res. Commun., 112: 284-289 (1983); Tilburn et al., Gene, 26: 205-221 (1983); Yelton et al., Proc. Natl. Acad. Sci. USA, 81: 1470-1474 (1984)) and A. niger (Kelly and Hynes, EMBO J., 4: 475-479 (1985)).

Host cells derived from multicellular organisms can also be used in the recombinant production of antibody or antibody fragment. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or

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invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6: 47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, 8: 277-279 (Plenum Publishing, 1986), and Maeda *et al.*, Nature, 315: 592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the antibody or antibody fragment DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding antibody or antibody fragment is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the antibody or antibody fragment DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1: 561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. See EP 321,196 published 21 June 1989.

Vertebrate cell culture is preferred for the recombinant production of full length antibodies. The propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years (Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)). Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol., 36: 59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77: 4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23: 243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci., 383: 44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells. Myeloma cells that do not otherwise produce immunoglobulin protein are also useful host cells for the recombinant production of full length antibodies.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for

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inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, CaPO₄ precipitation and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook et al., supra, is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with Agrobacterium tumefaciens is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23: 315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method described in sections 16.30-16.37 of Sambrook et al., supra, is preferred. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130: 946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76: 3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection, electroporation, or by protoplast fusion may also be used.

D. Culturing the Host Cells

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Prokaryotic cells used to produce the antibody or antibody fragment are cultured in suitable media as described generally in Sambrook et al., supra.

The mammalian host cells used to produce the antibody or antibody fragment can be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58: 44 (1979), Barnes and Sato, Anal. Biochem., 102: 255 (1980), U.S. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Pat. Re. 30,985; or U.S. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin TM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The host cells referred to in this disclosure encompass cells in in vitro culture as well as cells that

are within a host animal.

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E. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77: 5201-5205 (1980)), dot blotting (DNA analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ³²P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75: 734-738 (1980).

F. Purification of the Antibody or Antibody Fragment

In the case of a host cell secretion system, the antibody or antibody fragment is recovered from the culture medium. Alternatively, the antibody can be produced intracellularly, or produced in the periplasmic space of a bacterial host cell. If the antibody is produced intracellularly, as a first step, the host cells are lysed, and the resulting particulate debris is removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of E. coli. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand

depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABXTMresin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SepharoseTM chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g. from about 0-0.25M salt).

G. Production of Antibody Fragments

Various techniques have been developed for the production of the humanized antibody fragments of the invention, including Fab, Fab', Fab'-SH, or F(ab') 2 fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992) and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form F(ab') 2 fragments (Carter et al., Bio/Technology, 10:163-167 (1992)). According to another approach, F(ab') 2 fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner.

5. Uses of Anti-IL-8 Antibodies

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A. Diagnostic Uses

For diagnostic applications requiring the detection or quantitation of IL-8, the antibodies or antibody fragments of the invention typically will be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety can be a radioisotope, such as ³H, ¹⁴C, ³²P, ³⁵S, or ¹²⁵I; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; radioactive isotopic labels, such as, e.g., ¹²⁵I, ³²P, ¹⁴C, or ³H; or an enzyme, such as alkaline phosphatase, betagalactosidase, or horseradish peroxidase.

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Any method known in the art for separately conjugating the antibody or antibody fragment to the detectable moiety can be employed, including those methods described by Hunter et al., Nature 144:945 (1962); David et al., Biochemistry 13:1014 (1974); Pain et al., J. Immunol. Meth. 40:219 (1981); and Nygren, J. Histochem. and Cytochem. 30:407 (1982).

The antibodies and antibody fragments of the present invention can be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. For example, see Zola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard (which can be a IL-8 or an immunologically reactive portion thereof) to compete with the test sample analyte (IL-8) for binding with a limited amount of antibody or antibody fragment. The amount of IL-8 in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies or antibody fragments generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies can conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different antigenic portion, or epitope, of the protein (IL-8) to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex (U.S. Patent No. 4,376,110). The second antibody can itself be labeled with a detectable moiety (direct sandwich assays) or can be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme (e.g., horseradish peroxidase).

IL-8 antibodies and antibody fragments also are useful for the affinity purification of IL-8 from recombinant cell culture or natural sources. For example, these antibodies can be fixed to a solid support by techniques well known in the art so as to purify IL-8 from a source such as culture supernatant or tissue.

B. Therapeutic Compositions and Administration of Anti-IL-8 Antibody

The humanized anti-IL-8 antibodies and antibody fragments of the invention are useful in the treatment of inflammatory disorders, such as adult respiratory distress syndrome (ARDS), hypovolemic shock, ulcerative colitis, and rheumatoid arthritis.

Therapeutic formulations of the humanized anti-IL-8 antibodies and antibody fragments are prepared for storage by mixing the antibody or antibody fragment having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, supra), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins;

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hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

The humanized anti-IL-8 mAb or antibody fragment to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The humanized anti-IL-8 mAb or antibody fragment ordinarily will be stored in lyophilized form or in solution.

Therapeutic humanized anti-IL-8 mAb or antibody fragment compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of humanized anti-IL-8 mAb or antibody fragment administration is in accord with known methods, e.g., inhalation, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, by enema or suppository, or by sustained release systems as noted below. Preferably the antibody is given systemically or at a site of inflammation.

Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., Biopolymers 22:547 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167 (1981) and Langer, Chem. Tech. 12:98 (1982)), ethylene vinyl acetate (Langer et al., supra) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release humanized anti-IL-8 antibody or antibody fragment compositions also include liposomally entrapped antibody or antibody fragment. Liposomes containing an antibody or antibody fragment are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. U.S.A. 82:3688 (1985); Hwang et al., Proc. Natl. Acad. Sci. U.S.A. 77:4030 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamelar type in which the lipid content is greater than about 30 mole percent cholesterol, the selected proportion being adjusted for the most efficacious antibody or antibody fragment therapy.

An "effective amount" of the humanized anti-IL-8 antibody or antibody fragment to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the humanized anti-IL-8 antibody or antibody fragment until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

In the treatment and prevention of an inflammatory disorder with a humanized anti-IL-8 antibody

or antibody fragment of the invention, the antibody composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the antibody, the particular type of antibody, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat the inflammatory disorder, including treating acute or chronic respiratory diseases and reducing inflammatory responses. Such amount is preferably below the amount that is toxic to the host or renders the host significantly more susceptible to infections.

As a general proposition, the initial pharmaceutically effective amount of the antibody or antibody fragment administered parenterally per dose will be in the range of about 0.1 to 50 mg/kg of patient body weight per day, with the typical initial range of antibody used being 0.3 to 20 mg/kg/day, more preferably 0.3 to 15 mg/kg/day.

As noted above, however, these suggested amounts of antibody or antibody fragment are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above.

The antibody or antibody fragment need not be, but is optionally formulated with one or more agents currently used to prevent or treat the inflammatory disorder in question. For example, in rheumatoid arthritis, the antibody can be given in conjunction with a glucocorticosteroid. The effective amount of such other agents depends on the amount of antibody or antibody fragment present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

The following examples are offered by way of illustration and not by way of limitation. The disclosures of all references cited in the specification, and the disclosures of all citations in such references, are expressly incorporated herein by reference.

EXAMPLES

A. GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST HUMAN IL-8

Balb/c mice were immunized in each hind footpad or intraperitoneally with 10 μg of recombinant human IL-8 (produced as a fusion of (ser-IL-8)₇₂ with ubiquitin (Hebert *et al.* J. Immunology 145:3033-3040 (1990)); IL-8 is available commercially from PeproTech, Inc., Rocky Hill, NJ) resuspended in MPL/TDM (Ribi Immunochem. Research Inc., Hamilton, MT) and boosted twice with the same amount f IL-8. In these experiments, "IL-8" is intended to mean (ser-IL-8)₇₂ unless otherwise specified. A final boost of 10 μg of IL-8 was given 3 days before the fusion. Spleen cells or popliteal lymph node cells were fused with mouse myeloma P3X63Ag8U.1 (ATCC CRL1597), a non-secreting clone of the myeloma P3X63Ag8, using 35% polyethylene glycol as described before. Ten days after the fusion, culture supernatant was

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screened for the presence of monoclonal antibodies to IL-8 by ELISA.

The ELISA was performed as follows. Nunc 96-well immunoplates (Flow Lab, McLean, VA) were coated with 50 μl/well of 2 μg/ml IL-8 in phosphate-buffered saline (PBS) overnight at 4°C. The remaining steps were carried out at room temperature. Nonspecific binding sites were blocked with 0.5% bovine serum albumin (BSA) for 1 hour (hr). Plates were then incubated with 50 μl/well of hybridoma culture supernatants from 672 growing parental fusion wells for 1 hr, followed by the incubation with 50 μl/well of 1:1000 dilution of a 1 mg/ml stock solution of alkaline phosphatase-conjugated goat anti-mouse Ig (Tago Co., Foster City, CA) for 1 hr. The level of enzyme-linked antibody bound to the plate was determined by the addition of 100 μl/well of 0.5 mg/ml of r-nitrophenyl phosphate in sodium bicarbonate buffer, pH 9.6. The color reaction was measured at 405 nm with an ELISA plate reader (Titertrek Multiscan, Flow Lab, McLean, VA). Between each step, plates were washed three times in PBS containing 0.05% Tween 20.

Culture supernatants which promoted 4-fold more binding of IL-8 than did control medium were selected as positives. According to this criterion, 16 of 672 growing parental fusion wells (2%) were positive. These positive hybridoma cell lines were cloned at least twice by using the limiting dilution technique.

Seven of the positive hybridomas were further characterized as follows. The isotypes of the monoclonal antibodies were determined by coating Nunc 96-well immunoplates (Flow Lab, McLean, VA) with IL-8 overnight, blocking with BSA, incubating with culture supernatants followed by the addition of predetermined amount of isotype-specific alkaline phosphatase-conjugated goat anti-mouse lg (Fisher Biotech, Pittsburgh, PA). The level of conjugated antibodies bound to the plate was determined by the addition of r-nitrophenyl phosphate as described above.

All the monoclonal antibodies tested belonged to either $\lg G_1$ or $\lg G_2$ immunoglobulin isotype. Ascites fluid containing these monoclonal antibodies had antibody titers in the range of 10,000 to 100,000 as determined by the reciprocal of the dilution factor which gave 50% of the maximum binding in the ELISA.

To assess whether these monoclonal antibodies bound to the same epitopes, a competitive binding ELISA was performed. At a ratio of biotinylated mAb to unlabeled mAb of 1:100, the binding of biotinylated mAb 5.12.14 was significantly inhibited by its homologous mAb but not by mAb 4.1.3, while the binding of biotinylated mAb 4.1.3 was inhibited by mAb 4.1.3 but not by mAb 5.12.14. Monoclonal antibody 5.2.3 behaved similarly to mAb 4.1.3, while monoclonal antibodies 4.8 and 12.3.9 were similar to mAb 5.12.14. Thus, mAb 4.1.3 and mAb 5.2.3 bind to a different epitope(s) than the epitope recognized by monoclonal antibodies 12.3.9, 4.8 and 5.12.14.

Immunodot blot analysis was performed to assess antibody reactivity to IL-8 immobilized on nitrocellulose paper. All seven antibodies recognized IL-8 immobilized on paper, whereas a control mouse IgG antibody did not.

The ability of these monoclonal antibodies to capture soluble 125 I-IL-8 was assessed by a

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radioimmune precipitation test (RIP). Briefly, tracer ¹²⁵I-IL-8 (4 x 10⁴ cpm) was incubated with various dilutions of the monoclonal anti-IL-8 antibodies in 0.2 ml of PBS containing 0.5% BSA and 0.05% Tween 20 (assay buffer) for 1 hr at room temperature. One hundred microliters of a predetermined concentration of goat anti-mouse Ig antisera (Pel-Freez, Rogers, AR) were added and the mixture was incubated at room temperature for 1 hr. Immune complexes were precipitated by the addition of 0.5 ml of 6% polyethylene glycol (M.W. 8000) kept at 4°C. After centrifugation at 2,000 x g for 20 min at 4°C, the supernatant was removed by aspiration and the radioactivity remaining in the pellet was counted in a gamma counter. Percent specific binding was calculated as (precipitated cpm - background cpm)/ (total cpm - background cpm). Monoclonal antibodies 4.1.3, 5.2.3, 4.8, 5.12.14 and 12.3.9 captured ¹²⁵I-IL-8 very efficiently, while antibodies 9.2.4 and 8.9.1 were not able to capture soluble ¹²⁵I-IL-8 in the RIP even though they could bind to IL-8 coated onto ELISA plates (Table I).

The dissociation constants of these monoclonal antibodies were determined using a competitive binding RIP assay. Briefly, competitive inhibition of the binding each antibody to 125 I-IL-8 (20,000-40,000 cpm per assay) by various amounts of unlabeled IL-8 was determined by the RIP described above. The dissociation constant (affinity)of each mAb was determined by using Scatchard plot analysis (Munson, et al., Anal. Biochem. 107:220 (1980)) as provided in the VersaTerm-PRO computer program (Synergy Software, Reading, PA). The K_d 's of these monoclonal antibodies (with the exception of 9.2.4. and 8.9.1) were in the range from 2 x 10^{-8} to 3 x 10^{-10} M. Monoclonal antibodies tested (Table 3).

Table 3. Characterization of Anti-IL-8 Monoclonal Antibodies

Antibody	%Specific Binding to IL-8	K _d (M)	Isotype	pl
4.1.3	58	2 X 10 ⁻⁹	IgG ₁	4.3-6.1
5.2.3	34	2 X 10 ⁻⁸	IgG ₁	5.2-5.6
9.2.4	1	-	IgG ₁	7.0-7.5
8.9.1	2	-	IgG ₁	6.8-7.6

Antibody	%Specific Binding to 1L-8	K _d (M)	Isotype	pI
4.8	62	3 X 10 ⁻⁸	lgG _{2a}	6.1-7.1
5.12.14	98	3 X 10 ⁻¹⁰	IgG _{2a}	6.2-7.4
12.3.9	86	2 X 10 ⁻⁹	IgG _{2a}	6.5-7.1

To assess the ability of these monoclonal antibodies to neutralize IL-8 activity, the amount of 125 I-

IL-8 bound to human neutrophils in the presence of various amounts of culture supernatants and purified monoclonal antibodies was measured. Neutrophils were prepared by using Mono-Poly Resolving Medium (M-PRM) (Flow Lab. Inc., McLean, VA). Briefly fresh, heparinized human blood was loaded onto M-PRM at a ratio of blood to medium, 3.5:3.0, and centrifuged at 300 x g for 30 min at room temperature. Neutrophils enriched at the middle layer were collected and washed once in PBS. Such a preparation routinely contained greater than 95% neutrophils according to the Wright's Giemsa staining. The receptor binding assay was done as follows. 50 μl of ¹²⁵1-IL-8 (5 ng/ml) was incubated with 50 μl of unlabeled IL-8 (100 μg/ml) or monoclonal antibodies in PBS containing 0.1% BSA for 30 min at room temperature. The mixture was then incubated with 100 μl of neutrophils (10⁷ cells/ml) for 15 min at 37°C. The ¹²⁵1-IL-8 bound was separated from the unbound material by loading mixtures onto 0.4 ml of PBS containing 20% sucrose and 0.1% BSA and by centrifugation at 300 x g for 15 min. The supernatant was removed by aspiration and the radioactivity associated with the pellet was counted in a gamma counter.

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Monoclonal antibodies 4.1.3, 5.2.3, 4.8, 5.12.14, and 12.3.9 inhibited greater than 85% of the binding of IL-8 to human neutrophils at a 1:25 molar ratio of IL-8 to mAb. On the other hand, monoclonal antibodies 9.2.4 and 8.9.1 appeared to enhance the binding of IL-8 to its receptors on human neutrophils. Since a control mouse IgG also enhanced the binding of IL-8 on neutrophils, the enhancement of IL-8 binding to its receptors by mAb 9.2.4 and 8.9.1 appears to be nonspecific. Thus, monoclonal antibodies, 4.1.3, 5.1.3, 4.8, 5.12.14, and 12.3.9 are potential neutralizing monoclonal antibodies while monoclonal antibodies 8.9.1 and 9.2.4 are non-neutralizing monoclonal antibodies.

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The ability of the anti-IL-8 antibodies to block neutrophil chemotaxis induced by IL-8 was tested as follows. Neutrophil chemotaxis induced by IL-8 was determined using a Boyden chamber method

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(Larsen, et al. Science 243:1464 (1989)). One hundred μ1 of human neutrophils (10⁶ cells/m1) resuspended in RPM1-containing-0.1%-BSA-were-placed-in-the-upper-chamber and 29-μ1-of-the-IL-8-(20-nM)-with-or-without monoclonal antibodies were placed in the lower chamber. Cells were incubated for 1 hr at 37°C. Neutrophils migrated into the lower chamber were stained with Wright's Giemsa stain and counted under the microscope (100x magnification). Approximately 10 different fields per experimental group were examined. Neutralizing monoclonal antibodies 5.12.14 and 4.1.3 blocked almost 70% of the neutrophil chemotactic activity of IL-8 at 1:10 ratio of IL-8 to mAb.

The isoelectric focusing (IEF) pattern of each mAb was determined by applying purified antibodies on an IEF polyacrylamide gel (pH 3-9, Pharmacia) using the Fast gel system (Pharmacia, Piscataway, NJ). The IEF gel was pretreated with pharmalyte containing 1% Triton X100 (Sigma, St. Louis, MO) for 10 min before loading the samples. The IEF pattern was visualized by silver staining according to the instructions from the manufacturer. All of the monoclonal antibodies had different IEF patterns, confirming that they originated from different clones. The pl values for the antibodies are listed in Table 3.

All these monoclonal antibodies bound equally well to both (ala-IL-8)77 and (ser-IL-8)72 forms of IL-8. Because IL-8 has greater than 30% sequence homology with certain other members of the platelet factor 4 (PF4) family of inflammatory cytokines such as β-TG (Van Damme *et al.*, Eur. J. Biochem. 181:337(1989); Tanaka *et al.*, FEB 236(2):467 (1988)) and PF4 (Deuel *et al.*, Proc. Natl. Acad. Sci. U.S.A. 74:2256 (1977)), they were tested for possible cross reactivity to β-TG and PF4, as well as to another neutrophil activating factor, C5a. No detectable binding to any of these proteins was observed, with the exception of mAb 4.1.3, which had a slight cross reactivity to β-TG.

One of the antibodies, mAb 5.12.14, was further studied to determine whether it could block the IL-8 mediated release of elastase by neutrophils. Briefly, human neutrophils were resuspended in Hanks balanced salt solution (Gibco, Grand Island, NY) containing 1.0% BSA, Fraction V (Sigma, St. Louis, MO), 2 mg/ml alpha-D-glucose (Sigma), 4.2 mM sodium bicarbonate (Sigma) and 0.01 M HEPES, pH 7.1 (JRH Bioscience, Lenexa, KS). A stock of cytochalasin B (Sigma) was prepared (5 mg/ml in dimethylsulfoxide (Sigma) and stored at 2-8°C. Cytochalasin B was added to the neutrophil preparation to produce a final concentration of 5 µg/ml, and incubated for 15 min at 37°C. Human IL-8 was incubated with mAb 5.12.14 (20 µl), or a negative control antibody, in 1 ml polypropylene tubes (DBM Scientific, San Fernando, CA) for 30 min at 37°C. The final assay concentrations of IL-8 were 50 and 500 nM. The monoclonal antibodies were diluted to produce the following ratios (IL-8:Mab): 1:50, 1:10, 1:2, 1:1, and 1:0.25. Cytochalasin B-treated neutrophils were added (100 µl/tube) and incubated for 2 hours at 25°C. The tubes were centrifuged (210 X g, 2-8°C) for 10 min, and supernatants were transferred to 96 well tissue culture plates (30 µl/well). Elastase substrate stock, 10 mM methoxysuccinyl-alanyl-propyl-valyl-pnitroanilide (Calbiochem, La Jolla, CA) in DMSO was prepared and stored at 2-8°C. Elastase substrate solution (1.2 mM substrate, 1.2 M NaCl (Mallinckrodt, Paris, Kentucky), 0.12 M HEPES pH 7.2 in distilled water) was added (170 µl/well) to the supernatants and incubated for 0.5 to 2 hours at 37°C (until control

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O.D. of 1.0 was reached). Absorbance was measured at 405 nm (SLT 340 ATTC plate reader, SLT Lab Instruments, Austria).

The results are shown in Figure 1. At a 1:1 ratio of IL-8 to mAb 5.12.14, the antibody was able to effectively block the release of elastase from neutrophils.

The hybridoma producing antibody 5.12.14 was deposited on February 15, 1993 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATTC Accession No. HB 11553.

B. <u>GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST</u> RABBIT IL-8

Antibodies against rabbit IL-8 were generated in essentially the same process as anti-human IL-8 antibodies using rabbit IL-8 as immunogen (kindly provided by C. Broaddus; see also Yoshimura et al. <u>J. Immunol</u>. 146:3483 (1991)). The antibody was characterized as described above for binding to other cytokines coated onto ELISA plates; no measurable binding was found to MGSA, fMLP, C5a, b-TG, TNF, PF4, or IL-1.

The hybridoma producing antibody 6G4.2.5 was deposited on September 28, 1994, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATTC Accession No. HB 11722.

Recombinant human-murine chimeric Fabs for 5.12.14 and 6G4.2.5 were constructed as described below. A chimeric 6G.4.25 Fab is compared with a chimeric 5.12.14 Fab in detail below.

1. INHIBITION OF IL-8 BINDING TO HUMAN NEUTROPHILS BY 5.12.14-FAB AND 6G4 2.5-FAB

The ability of the two chimeric Fabs, 5.12.14-Fab and 6G4.2.5-Fab, to efficiently bind IL-8 and prevent IL-8 from binding to IL-8 receptors on human neutrophils was determined by performing a competition binding assay which allows the calculation of the IC $_{50}$ - concentration required to achieve 50% inhibition of IL-8 binding.

Human neutrophils (5 X 10⁵) were incubated for 1 hour at 4°C with 0.5nM ¹²⁵I-IL-8 in the presence of various concentrations (0 to 300 nM) of 5.12.14-Fab, 6G4.2.5-Fab, an isotype control (4D5-Fab) or unlabeled IL-8. After the incubation, the unbound ¹²⁵I-IL-8 was removed by centrifugation through a solution of 20% sucrose and 0.1% bovine serum albumin in phosphate buffered saline and the amount of

demonstrates the inhibition of ¹²⁵I-IL-8 binding to neutrophils by unlabeled IL-8. Figure 3 demonstrates that a negative isotype matched Fab does not inhibit the binding of ¹²⁵I-IL-8 to human neutrophils. Both the anti-IL-8 Fabs, 5.12.14 Fab (Figure 4) and 6G.4.25 Fab (Figure 5) were able to inhibit the binding of ¹²⁵I-IL-8 to human neutrophils with an average IC₅₀ of 1.6 nM and 7.5 nM, respectively.

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2. <u>INHIBITION OF IL-8-MEDIATED NEUTROPHIL CHEMOTAXIS BY 5.12.14-FAB AND 6G4.2.5-FAB</u>

Human neutrophils were isolated, counted and resuspended at 5×10^6 cells/ml in Hank's balanced salt solution (abbreviated HBSS; without calcium and magnesium) with 0.1% bovine serum albumin. The neutrophils were labeled by adding calcein AM (Molecular Probe, Eugene, OR) at a final concentration of 2.0 μ M. Following a 30 minute incubation at 37°C, cells were washed twice with HBSS-BSA and resuspended at 5×10^6 cells/ml.

Chemotaxis experiments were carried out in a Neuro Probe (Cabin John, MD) 96-well chamber, model MBB96. Experimental samples (buffer only control, IL-8 alone or IL-8 + Fabs) were loaded in a Polyfiltronics 96-well View plate (Neuro Probe Inc.) placed in the lower chamber. 100 µl of the calcein AM-labeled neutrophils were added to the upper chambers and allowed to migrate through a 5 micrometer porosity PVP free polycarbonate framed filter (Neuro Probe Inc.) toward the bottom chamber sample. The chemotaxis apparatus was then incubated for 40 to 60 minutes at 37°C with 5% CO₂. At the end of the incubation, neutrophils remaining in the upper chamber were aspirated and upper chambers were washed three times with PBS. Then the polycarbonate filter was removed, non-migrating cells were wiped off with a squeegee wetted with PBS, and the filter was air dried for 15 minutes.

The relative number of neutrophils migrating through the filter (Neutrophil migration index) was determined by measuring fluorescence intensity of the filter and the fluorescence intensity of the contents of the lower chamber and adding the two values together. Fluorescence intensity was measured with a CytoFluor 2300 fluorescent plate reader (Millipore Corp. Bedford, MA) configured to read a Corning 96-well plate using the 485-20 nm excitation filter and a 530-25 emission filter, with the sensitivity set at 3.

The results are shown in Figures 6 and 7. Figure 6 demonstrates the inhibition of human IL-8 mediated neutrophil chemotaxis by chimeric 6G4.2.5 and 5.12.14 Fabs. Figure 7 demonstrates the relative abilities of chimeric 6G4.2.5 and 5.12.14 Fabs to inhibit rabbit IL-8 mediated neutrophil chemotaxis.

3. <u>INHIBITION OF IL-8-MEDIATED NEUTROPHIL ELASTASE RELEASE BY VARIOUS</u> CONCENTRATIONS OF 6G4.2.5 AND 5.12.14 FABS

Blood was drawn from healthy male donors into heparinized syringes. Neutrophils were isolated by dextran sedimentation, centrifugation over Lymphocyte Separation Medium (Organon Teknika, Durham, NC), and hypotonic lysis of contaminating red blood cells as described by Berman *et al.* (J. Cell Biochem. 52:183 (1993)). The final neutrophil pellet was suspended at a concentration of 1 x 10⁷ cells/ml in assay buffer, which consisted of Hanks Balanced Salt Solution (GIBCO, Grand Island, NY) supplemented with 1.0% BSA (fraction V, Sigma, St. Louis, MO), 2 mg/ml glucose, 4.2 mM sodium bicarbonate, and 0.01 M HEPES, pH 7.2. The neutrophils were stored at 4°C for not longer than 1 hr.

IL-8 (10 µI) was mixed with anti-IL-8 Fab, an isotype control Fab, or buffer (20 µI) in 1 ml polypropylene tubes and incubated in a 37°C water bath for 30 min. IL-8 was used at final concentrations ranging from 0.01 to 1000 nM in dose response studies (Figure 8) and at a final concentration of 100 nM in

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the experiments addressing the effects of the Fabs on elastase release (Figures 9 and 10). Fab concentrations ranged from approximately 20 nM to 300 nM, resulting in Fab:IL-8 molar ratios of 0.2:1 to 3:1. Cytochalasin B (Sigma) was added to the neutrophil suspension at a concentration of 5 µg/ml (using a 5 mg/ml stock solution made up in DMSO), and the cells were incubated for 15 min in a 37°C water bath. Cytochalasin B-treated neutrophils (100 µl) were then added to the IL-8/Fab mixtures. After a 3 hr incubation at room temperature, the neutrophils were pelleted by centrifugation (200 x g for 5 min), and aliquots of the cell-free supernatants were transferred to 96 well plates (30 µl/well). The elastase substrate, methoxysuccinyl-alanyl-prolyl-valyl-p-nitroanilide (Calbiochem, La Jolla, CA), was prepared as a 10 mM stock solution in DMSO and stored at 4°C. Elastase substrate working solution was prepared just prior to use (1.2 mM elastase substrate, 1.2 M NaCl, 0.12 M HEPES, pH 7.2), and 170 µl was added to each sample-containing well. The plates were placed in a 37°C tissue culture incubator for 30 min or until an optical density reading for the positive controls reached at least 1.0. Absorbance was measured at 405 nm using an SLT 340 plate reader (SLT Lab Instruments, Austria).

Figure 9 demonstrates the ability of the chimeric anti-IL-8 Fabs to inhibit elastase release from human neutrophils stimulated by human IL-8; Figure 10 demonstrates the relative abilities of the chimeric anti-IL-8 Fabs to inhibit elastase release from human neutrophils stimulated by rabbit IL-8.

C. MOLECULAR CLONING OF THE VARIABLE LIGHT AND HEAVY REGIONS OF THE MURINE 5.12.14 (ANTI-IL-8) MONOCLONAL ANTIBODY

Total RNA was isolated from 1 X 10⁸ cells (hybridoma cell line ATCC HB-11722) using the procedure described by Chomczynski and Sacchi (Anal. Biochem. 162:156 (1987)). First strand cDNA was synthesized by specifically priming the mRNA with synthetic DNA oligonucleotides designed to hybridize with regions of the murine RNA encoding the constant region of the kappa light chain or the IgG2a heavy chain (the DNA sequence of these regions are published in Sequences of Proteins of Immunological Interest, Kabat, E. A. et al. (1991) NIH Publication 91-3242, V 1-3.). Three primers (SEQ ID NOS: 1-6) were designed for each of the light and heavy chains to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis (Figure 13). Amplification of the first strand cDNA to doublestranded (ds) DNA was accomplished using two sets of synthetic DNA oligonucleotide primers: one forward primer (SEQ ID NOS: 7-9) and one reverse primer (SEQ ID NO: 10) for the light chain variable region amplification (Figure 14) and one forward primer (SEQ ID NOS: 11-14) and one reverse primer (SEQ ID NOS: 15-18) for the heavy chain variable region amplification (Figure 15). The N-terminal sequence of the first eight amino acids of either the light or heavy chains of 5.12.14 was used to generate a putative murine DNA sequence corresponding to this region. (A total of 29 amino acids was sequenced from the N-terminus of both the light chain and heavy chain variable regions using the Edman degradation protein sequencing technique.) This information was used to design the forward amplification primers which were made degenerate in the third position for some codons to increase the chances of primer hybridization to the natural murine DNA codons and also included the unique restriction site, MluI, for both the light chain variable region forward primer and the heavy chain variable region forward primer to

facilitate ligation to the 3' end of the STII element in the cloning vector. The reverse amplification primers were designed to anneal with the murine DNA sequence corresponding to a portion of the constant region of the light or heavy chains near the variable/constant junction. The light chain variable region reverse primer contained a unique BstBI restriction site and the heavy chain variable region reverse primer contained a unique ApaI restriction site for ligation to the 5' end of either the human IgG1 constant light or IgG1 constant heavy regions in the vectors, pB13.1 (light chain) and pB14 (heavy chain). The polymerase chain reaction using these primer sets yielded DNA fragments of approximately 400 bp. The cDNA encoding the 5.12.14 light chain variable region was cloned into the vector pB13.1, to form pA51214VL and the 5.12.14 heavy chain variable region was cloned into the vector, pB14, to form pA51214VH. The cDNA inserts were characterized by DNA sequencing and are presented in the DNA sequence (SEQ ID NO: 19) and amino acid sequence (SEQ ID NO: 20) of Figure 16 (murine light chain variable region) and in the DNA sequence (SEQ ID NO: 21) and amino acid (SEQ ID NO: 22) of Figure 17 (murine heavy chain variable region).

D. CONSTRUCTION OF A 5.12.14 FAB VECTOR

In the initial construct, pA51214VL, the amino acids between the end of the 5.12.14 murine light chain variable sequence and the unique cloning site, BstBI, in the human IgG1 constant light sequence were of murine origin corresponding to the first 13 amino acids of the murine IgG1 constant region (Figure 16). Therefore, this plasmid contained a superfluous portion of the murine constant region separating the 5.12.14 murine light chain variable region and the human light chain lgG1 constant region. This intervening sequence would alter the amino acid sequence of the chimera and most likely produce an incorrectly folded Fab. This problem was addressed by immediately truncating the cDNA clone after A109 and re-positioning the BstBI site to the variable/constant junction by the polymerase chain reaction. Figure 18 shows the amplification primers used to make these modifications. The forward primer, VL.front (SEQ ID NO: 23), was designed to match the last five amino acids of the STII signal sequence, including the MluI cloning site, and the first 4 amino acids of the 5.12.14 murine light chain variable sequence. The sequence was altered from the original cDNA in the third position of the first two codons D1 (T to C) and I2 (C to T) to create a unique EcoRV cloning site which was used for later constructions. The reverse primer, VL.rear (SEQ ID NO: 24), was designed to match the first three amino acids of the human IgG1 constant light sequence and the last seven amino acids of the 5.12.14 light chain variable sequence which included a unique BstBI cloning site. In the process of adding the BstBI site, the nucleotide sequence encoding several amino acids were altered: L106 (TTG to CTT), K107 (AAA to CGA) resulting in a conservative amino acid substitution to arginine, and R108 (CGG to AGA). The PCR product encoding the modified 5.12.14 light chain variable sequence was then subcloned into pB13.1 in a two-part ligation. The MluI-BstBI digested 5.12.14 PCR product encoding the light chain variable region was ligated into MluI-BstBI digested vector to form the plasmid, pA51214VL'. The modified cDNA was characterized by DNA sequencing. The coding sequence for the 5.12.14 light chain is shown in Figure 19.

Likewise, the DNA sequence between the end of the heavy chain variable region and the unique

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cloning site, Apal, in the human IgG1 heavy chain constant domain of pA51214VH was reconstructed to change_the_amino_acids_in_this_area_fr m_murine to human. This was done by the polymerase chain reaction. Amplification of the murine 5.12.14 heavy chain variable sequence was accomplished using the primers shown in Figure 18. The forward PCR primer (SEQ ID NO: 25) was designed to match nucleotides 867-887 in pA51214VH upstream of the STII signal sequence and the putative cDNA sequence encoding the heavy chain variable region and included the unique cloning site SpeI. The reverse PCR primer (SEQ ID NO: 26) was designed to match the last four amino acids of the 5.12.14 heavy chain variable sequence and the first six amino acids corresponding to the human IgG1 heavy constant sequence which also included the unique cloning site, ApaI. The PCR product encoding the modified 5.12.14 heavy chain variable sequence was then subcloned to the expression plasmid, pMHM24.2.28 in a two-part ligation. The vector was digested with SpeI-ApaI and the SpeI-ApaI digested 5.12.14 PCR product encoding the heavy chain variable region was ligated into it to form the plasmid, pA51214VH'. The modified cDNA was characterized by DNA sequencing. The coding sequence (SEQ ID NO: 29) and amino acid sequence (SEQ ID NO: 30) of Figures 20A-20B.

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The first expression plasmid, pantilL-8.1, encoding the chimeric Fab of 5.12.14 was made by digesting pA51214VH' with EcoRV and Bpu11021 to replace the EcoRV-Bpu1102I fragment with a EcoRV-Bpu1102I fragment encoding the murine 5.12.14 light chain variable region of pA51214VL'. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 5.12.14.

Preliminary analysis of Fab expression using pantilL-8.1 showed that the light and heavy chains were produced intracellularly but very little was being secreted into the periplasmic space of <u>E. coli</u>. To correct this problem, a second expression plasmid was constructed.

The second expression plasmid, pantilL-8.2, was constructed using the plasmid, pmy187, as the vector. Plasmid pantilL-8.2 was made by digesting pmy187 with MluI and SphI and the MluI (partial)-SphI fragment encoding the murine 5.12.14 murine-human chimeric Fab of pantilL-8.1 was ligated into it. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 5.12.14.

The plasmid pantiIL-8.2 was deposited on February 10, 1995 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATTC Accession No. ATCC 97056.

E. MOLECULAR CLONING OF THE VARIABLE LIGHT AND HEAVY REGIONS OF THE MURINE 6G4.2.5 MONOCLONAL ANTIBODY

Total RNA was isolated from 1x10⁸ cells (hybridoma cell line 6G4.2.5) using the procedure described by Chomczynski and Sacchi (Anal. Biochem. 162:156 (1987)). First strand cDNA was synthesized by specifically priming the mRNA with synthetic DNA oligonucleotides designed to hybridize with regions of the murine RNA encoding the constant region of the kappa light chain or the IgG2a heavy chain (the DNA sequence of these regions are published in Sequences of Proteins of Immunological Interest,

Kabat et al. (1991) NIH Publication 91-3242, V 1-3). Three primers (SEQ ID NOS: 31-36) were designed for each the light and heavy chains to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis (Figure 21). Amplification of the first strand cDNA to double-stranded (ds) DNA was accomplished using two sets of synthetic DNA oligonucleotide primers: one forward primer (SEQ ID NOS: 37-39) and one reverse primer (SEQ ID NO: 40) for the light chain variable region amplification (Figure 22) and one forward primer (SEQ ID NOS: 41-42) and one reverse primer (SEQ ID NOS: 43-46) for the heavy chain variable region amplification (Figure 23). The N-terminal sequence of the first eight amino acids of either the light or heavy chains of 6G4.2.5 was used to generate a putative murine DNA sequence corresponding to this region. (A total of 29 amino acids were sequenced from the N-terminus of both the light chain and heavy chain variable regions using the Edman degradation protein sequencing technique.) This information was used to design the forward amplification primers which were made degenerate in the third position for some codons to increase the chances of primer hybridization to the natural murine DNA codons and also included the unique restriction site, Nsil, for the light chain variable region forward primer and the unique restriction site, MluI, for the heavy chain variable region forward primer to facilitate ligation to the 3' end of the STII element in the vector, pchimFab. The reverse amplification primers were designed to anneal with the murine DNA sequence corresponding to a portion of the constant region of the light or heavy chains near the variable/constant junction. The light chain variable region reverse primer contained a unique MunI restriction site and the heavy chain variable region reverse primer contained a unique Apal restriction site for ligation to the 5' end of either the human IgG1 constant light or IgG1 constant heavy regions in the vector, pchimFab. The polymerase chain reaction using these primer sets yielded DNA fragments of approximately 400 bp and were cloned individually into the vector, pchimFab, to form p6G425VL and p6G425VH. The cDNA inserts were characterized by DNA sequencing and are presented in the DNA sequence (SEQ ID NO: 47) and amino acid sequence (SEQ ID NO: 48) of Figure 24 (murine light chain variable region) and the DNA sequence (SEQ ID NO: 49) and amino acid sequence (SEQ ID NO: 50) of Figure 25 (murine heavy chain variable region).

F. CONSTRUCTION OF A 6G4.2.5 CHIMERIC FAB VECTOR

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In the initial construct, p6G425VL, the amino acids between the end of the 6G4.2.5 murine light chain variable sequence and the unique cloning site, MunI, in the human IgG1 constant light sequence were of murine origin. These amino acids must match the human IgG1 amino acid sequence to allow proper folding of the chimeric Fab. Two murine amino acids, D115 and S121, differed dramatically from the amino acids found in the loops of the β-strands of the human IgG1 constant domain and were converted to the proper human amino acid residues, V115 and F121, by site-directed mutagenesis using the primers (SEQ ID NOS: 51-54) shown in Figure 26. These specific mutations were confirmed by DNA sequencing and the modified plasmid named p6G425VL'. The coding sequence is shown in the DNA sequence (SEQ ID NO: 55) and amino acid sequence (SEQ ID NO: 56) of Figures 27A-27B.

Likewise, the DNA sequence between the end of the heavy chain variable region and the unique cloning site, Apal, in the human IgG1 heavy chain constant domain of p6G425VH was reconstructed to

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change the amino acids in this area from murine to human. This process was facilitated by the discovery of a-BstEII-site-near-the-end-of-the-heavy-chain-variable region. This site and the ApaI site were used for the addition of a synthetic piece of DNA encoding the corresponding IgG human amino acid sequence. The synthetic oligo-nucleotides shown in Figure 26 were designed as complements of one another to allow the formation of a 27 bp piece of ds DNA. The construction was performed as a three-part ligation because the plasmid, p6G425VH, contained an additional BstEII site within the vector sequence. A 5309 bp fragment of p6G425VH digested with MluI-ApaI was ligated to a 388 bp fragment carrying the 6G4.2.5 heavy chain variable region and a 27 bp synthetic DNA fragment encoding the first six amino acids of the human IgGI constant region to form the plasmid, p6G425VH. The insertion of the synthetic piece of DNA was confirmed by DNA sequencing. The coding sequence is shown in the DNA sequence (SEQ ID NO: 57) and amino acid sequence (SEQ ID NO: 58) of Figures 28A-28B.

The expression plasmid, p6G425chim2, encoding the chimeric Fab of 6G4.2.5 was made by digesting p6G425chimVL' with MluI and ApaI to remove the STII-murine HPC4 heavy chain variable region and replacing it with the MluI-ApaI fragment encoding the STII-murine 6G4.2.5 heavy chain variable region of p6G425chimVH'. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 6G4.2.5.

The plasmid p6G425chim2 was deposited on February 10, 1995 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATTC Accession No. 97055.

20 G. CONSTRUCTION OF HUMANIZED VERSIONS OF ANTI-IL-8 ANTIBODY 6G4.2.5

The murine cDNA sequence information obtained from the hybridoma cell line, 6G4.2.5, was used to construct recombinant humanized variants of the murine anti-IL-8 antibody. The first humanized variant, F(ab)-1, was made by grafting synthetic DNA oligonucleotide primers encoding the murine CDRs of the heavy and light chains onto a phagemid vector, pEMX1 (Werther et al., J. Immunol, 157: 4986-4995 (1996)), which contains a human 6-subgroup I light chain and a human IgG1 subgroup III heavy chain (Fig. 29). Amino acids comprising the framework of the antibody that were potentially important for maintaining the conformations necessary for high affinity binding to IL-8 by the complementarity-determining regions (CDR) were identified by comparing molecular models of the murine and humanized 6G4.2.5 (F(ab)-1) variable domains using methods described by Carter et al., PNAS 89:4285 (1992) and Eigenbrot, et. al., J. Mol. Biol. 229:969 (1993). Additional humanized framework variants (F(ab) 2-9) were constructed from the information obtained from these models and are presented in Table 4 below. In these variants, the sitedirected mutagenesis methods of Kunkel, Proc. Natl. Acad. Sci USA), 82:488 (1985) were utilized to exchange specific human framework residues with their corresponding 6G4.2.5 murine counterparts. Subsequently, the entire coding sequence of each variant was confirmed by DNA sequencing. Expression and purification of each F(ab) variant was performed as previously described by Werther et. al., supra, with the exception that hen egg white lysozyme was omitted from the purification pr toc l. The variant antibodies were analyzed by SDS-PAGE, electrospray mass spectroscopy and amino acid analysis.

Tabl 4 - Humanized 6G425 Variants

IC50°

Variant	Version	Template	Changes ^a	Purpose ^b	Mean	S.D.	N
F(ab)-1	version 1		CDR Swap		63.0	12.3	4
F(ab)-2	version 2	F(ab)-1	PheH67 <i>Ala</i>	packaging w/ CDR H2	106.0	17.0	2
F(ab)-3	version 3	F(ab)-1	ArgH71 <i>Val</i>	packaging w/ CDRs H1, H2	79.8	42.2	4
F(ab)-4	version 6	F(ab)-1	IleH69 <i>Leu</i>	packaging w/ CDR H2	44.7	9.0	3
F(ab)-5	version 7	F(ab)-1	LeuH78 <i>Ala</i>	packaging w/ CDRs H1, H2	52.7	31.0	9
F(ab)-6	version 8	F(ab)-1	IleH69 <i>Leu</i> LeuH78 <i>Ala</i>	combine F(ab)- 4 and -5	34.6	6.7	7
F(ab)-7	version 16	F(ab)-6	LeuH80Val	packaging w/ CDR H1	38.4	9.1	2
F(ab)-8	version 19	F(ab)-6	ArgH38Lys	packaging w/ CDR H2	14.0	5.7	2
F(ab)-9	version 11	F(ab)-6	GluH6 <i>Gln</i>	packaging w/ CDR H3	19.0	5.1	7
Chimeric ^d F(ab)					11.4	7.0	13
rhu4D5° F(ab)					>200µM		5

- Amino acid changes made relative to the template used. Murine residues are in bold italics and residue numbering is according to Kabat et al.
 - b Purpose for making changes based upon interactions observed in molecular models of the humanized and murine variable domains.
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 c nM concentration of variant necessary to inhibit binding of iodinated IL-8 to human neutrophils in the competitive binding assay.
- d Chimeric F(ab) is a (F(ab) which carries the murine heavy and light chain variable domains fused to the human light chain kI constant domain and the human heavy chain subgroup III constant domain I respectively.

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e. rhu4D5F(ab) is of the same isotype as the humanized 6G425 F(ab)s and is a humanized anti-HER2 F(ab) and therefore should not bind to IL8.

The first humanized variant, F(ab)-1, was an unaltered CDR swap in which all the murine CDR amino acids defined by both x-ray crystallography and sequence hypervariability were transferred to the human framework. When the purified F(ab) was tested for its ability to inhibit 125 I-IL-8 binding to human neutrophils according to the methods described in Section (B)(1) above, a 5.5 fold reduction in binding affinity was evident as shown in Table 4 above. Subsequent versions of F(ab)-1 were engineered to fashion the 3-dimensional structure of the CDR loops into a more favorable conformation for binding IL-8. The relative affinities of the F(ab) variants determined from competition binding experiments using human neutrophils as described in Section (B)(1) above are presented in Table 4 above. A slight decrease in IL-8 binding (<2 fold) was observed for F(ab)-2-3 while only slight increases in IL-8 binding were noted for F(ab)3-5. Variant F(ab)-6 had the highest increase in affinity for IL-8 (approximately 2 fold), exhibiting an IL-8 binding affinity of 34.6nM compared to the F(ab)-1 IL-8 binding affinity of 63nM. The substitutions of murine Leu for Ile at H69 and murine Ala for Leu at H78 are predicted to influence the packing of CDRs H1 and H2. Further framework substitutions using the F(ab)-6 variant as template were made to bring the binding affinity closer to that of the chimeric F(ab). In-vitro binding experiments revealed no change in affinity for F(ab)-7 (38.4nM) but a significant improvement in affinity for F(ab)-8/9 of 14nM and 19 nM, respectively. By analysis of a 3-D computer-generated model of the anti-IL-8 antibody, it was hypothesized that the substitution of murine Lys for Arg at H38 in F(ab)-8 influences CDR-H2 while a change at H6 of murine Gln for Glu in F(ab)-9 affects CDR-H3. Examination of the human antibody sequences with respect to amino acid variability revealed that the frequency of Arg at residue H38 is >99% whereas residue H6 is either Gln ~20% or Glu ~80% (Kabat et. al., Sequences of Proteins of Immunological Interest 5th Ed. (1991)). Therefore, to reduce the likelihood of causing an immune response to the antibody, F(ab)-9 was chosen over F(ab)-8 for further affinity maturation studies. Variant F(ab)-9 was also tested for its ability to inhibit IL-8-mediated chemotaxis (Fig. 30). This antibody was able to block neutrophil migration induced by wild-type human IL-8, human monomeric IL-8 and Rhesus IL-8 with IC₅₀=s of approximately 12nM, 15nM, and 22nM, respectively, in IL-8 mediated neutrophil chemotaxis inhibition assays performed as described in Section (B)(2) above. The amino acid sequence for variant F(ab)-8 is provided in Fig. 31c. The F(ab)-8 was found to block human and rhesus IL-8-mediated chemotaxis with IC50=s of 12nM and

10nM, respectively, in 1L-8 mediated neutrophil chemotaxis inhibition assays performed as described in Section (B)(2) above.

H. <u>CONSTRUCTION OF AN ANTI-IL-8-GENE III FUSION PROTEIN FOR PHAGE DISPLAY AND ALANINE SCANNING MUTAGENESIS</u>

An expression plasmid, pPh6G4.V11, encoding a fusion protein (heavy chain of the humanized 6G4.2.5 version 11 antibody and the M13 phage gene-III coat protein) and the light chain of the humanized 6G4.2.5 version 11 antibody was assembled to produce a monovalent display of the anti-IL-8 antibody on

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phage particles. The construct was made by digesting the plasmid, pFPHX, with EcoRV and Apal to remove the existing irrelevant antibody coding sequence and replacing it with a 1305bp EcoRV-Apal fragment from the plasmid, p6G4.V11, encoding the humanized 6G4.2.5 version 11 anti-IL-8 antibody. The translated sequence of the humanized 6G4.2.5 version 11 heavy chain (SEQ ID NO: 66), peptide linker and gene III coat protein (SEQ ID NO: 67) is shown in Fig. 31A. The pFPHX plasmid is a derivative of phGHam-3 which contains an in-frame amber codon (TAG) between the human growth hormone and gene-III DNA coding sequences. When transformed into an amber suppressor strain of *E*. coli, the codon (TAG) is read as Glutamate producing a growth hormone (hGH)-gene III fusion protein. Likewise, in a normal strain of *E*. coli, the codon (TAG) is read as a stop preventing translational read-through into the gene-III sequence and thus allowing the production of soluble hGH. The pGHam-3 plasmid is described in Methods: A Companion to Methods in Enzymology, 3:205 (1991). The final product, pPh6G4.V11, was used as the template for the alanine scanning mutagenesis of the CDRs and for the construction of randomized CDR libraries of the humanized 6G4.V11 antibody.

1. ALANINE SCANNING MUTAGENESIS OF HUMANIZED ANTIBODY 6G4.2.5 VERSION 11

The solvent exposed amino acid residues in the CDRs of the humanized anti-IL-8 6G4.2.5 version 11 antibody (h6G4V11) were identified by analysis of a 3-D computer-generated model of the anti-IL-8 antibody. In order to determine which solvent exposed amino acids in the CDRs affect binding to interleukin-8, each of the solvent exposed amino acids was individually changed to alanine, creating a panel of mutant antibodies wherein each mutant contained an alanine substitution at a single solvent exposed residue. The alanine scanning mutagenesis was performed as described by Leong et. al., J. Biol. Chem., 269: 19343 (1994)).

The IC₅₀'s (relative affinities) of h6G4V11 wt and mutated antibodies were established using a Competition Phage ELISA Assay described by Cunningham et. al., (EMBO J. 13:2508 (1994)) and Lee et. al., (Science 270:1657 (1995)). The assay measures the ability of each antibody to bind IL-8 coated onto a 96-well plate in the presence of various concentrations of free IL-8 (0.2 to 1uM) in solution. The first step of the assay requires that the concentrations of the phage carrying the wild type and mutated antibodies be normalized, allowing a comparison of the relative affinities of each antibody. The normalization was accomplished by titering the phage on the IL-8 coated plates and establishing their EC50. Sulfhydryl coated 96-well binding plates (Corning-Costar; Wilmington, MA) were incubated with a 0.1mg/ml solution of K64C IL-8 (Lysine 64 is substituted with Cysteine to allow the formation of a disulfide bond between the free thiol group of K64C IL-8 and the sulfhydryl coated plate, which results in the positioning of the IL-8 receptor binding domains towards the solution interface) in phosphate buffered saline (PBS) pH 6.5 containing 1mM EDTA for 1 hour at 25EC followed by three washes with PBS and a final incubation with a solution of PBS containing 1.75mg/ml of L-cysteine-HCl and 0.1M NaHCO₃ to block any free reactive sulfhydryl groups on the plate. The plates were washed once more and stored covered at 4EC with 200ul of PBS/well. Phage displaying either the reference antibody, h6G4V11, or the mutant h6G4V11 antibodies were grown and harvested by PEG precipitation. The phage were resuspended in 500ul 10mM Tris-HCl pH

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7.5, 1mM EDTA and 100mM NaCl and held at 4EC for no longer than 3 hours. An aliquot of each phage was diluted 4-fold in PBS containing 0.05% Tween-20 (BioRad, Richmond, Ca.) and 0.5% BSA RIA grade (Sigma, St. Louis, Mo.) (PBB) and added to IL-8 coated plates blocked for at least 2 hours at 25EC with 50mg/ml skim milk powder in 25mM Carbonate Buffer pH 9.6. The phage were next serially diluted in 3 fold steps down the plate from well A through H. The plates were incubated for 1 hour at 25EC followed by nine quick washes with PBS containing 0.05% Tween-20 (PBST). The plates were then incubated with a 1:3200 dilution of rabbit anti-phage antibody and a 1:1600 dilution of secondary goat-anti-rabbit Fc HRPconjugated antibody for 15 minutes at 25EC followed by nine quick washes with PBST. The plates were developed with 80ul/well of 1mg/ml OPD (Sigma, St. Louis, Mo) in Citrate Phosphate buffer pH 5.0 containing 0.015% H₂O₂ for 4 minutes at 25EC and the reaction stopped with the addition of 40ul of 4.5M H₂SO₄. The plates were analyzed at wavelength 8₄₉₂ in a SLT model 340ATTC plate reader (SLT Lab The individual EC₅₀=s were determined by analyzing the data using the program Kaleidagraph (Synergy Software, Reading, Pa.) and a 4-parameter fit equation. The phage held at 4EC were then immediately diluted in PBB to achieve a final concentration corresponding to their respective EC50 or target OD492 for the competition segment of the experiment, and dispensed into a 96 well plate containing 4-fold serial dilutions of soluble IL-8 ranging from luM in well A and ending with 0.2uM in well H. Using a 12-channel pipet, 100ul of the phage/IL-8 mixture was transferred to an IL-8 coated 96-well plate and executed as described above. Each sample was done in triplicate - 3 columns/sample.

Table 5 - Relative Affinities (IC50) for Alanine-scan Anti-IL-8 6G4V11 CDR Mutants

CDR	Amino Acid Residue	Avg IC50 (nM)	Std Dev
V11	Reference	11.5	6.4
CDR-L1	S26	6.3	2.9
	Q27	10.2	2.4
	S28	14.2	5.2
	V30	29.1	12.3
	H31	580.3	243.0
	133	64.2	14.6
	N35	3.3	0.7
	T36	138.0	nd
	Y37	NDB	nd
CDR-L2	K55	24.2	14.9
	V56	15.5	3.8
	S57	12.4	4.0
	N58	17.6	3.7
	R59	nd	nd
CDR-L3	S96	10.8	4.4
	T97	70.6	55.2

CDR	Amino Acid Residue	Avg IC50 (nM)	Std Dev
	H98		1.2
	V99	19.6	1.9
CDR-H1	S28	8.6	3.1
	S30	nd	nd
	S31	7.8	2.5
	H32	13.3	5.8
	Y53	48.2	15.8
CDR-H2	Y50	35.6	13.0
	D52	13.3	7.5
	S53	6.0	3.4
	N54	96.0	5.8
	E56	15.8	4.5
	T57	8.4	1.6
	T58	11.3	1.8
	Y59	9.1	3.7
	Q61	12.6	6.4
	K64	18.5	12.1
CDR-H3	D96	NDB	nd
	Y97	NDB	nd
	R98	36.6	15.3
	Y99	199.5	nd
	N100	278.3	169.4
	D102	159.2	44
	W103	NDB	nd
	F104	NDB	nd
	F105	209.4	72.3
	D106	25.3	21.7

Each sample performed in triplicate/experiment.

NDB = No Detectable Binding /nd = value not determined*

Residue numbering is according to Kabat et al.

The results of the alanine-scan are summarized in Table 5 above. The alanine substitutions in of many of the mutant antibodies had little or no adverse effects (<3 fold) on the binding affinity for IL-8. Mutants that were found to exhibit no detectable binding of IL-8 (NDB) presumably contained disruptions in the conformational structure of the antibody conferred by crucial structural or buried amino acids in the CDR. Based on the results of the scan, CDR-H3 (heavy chain, 3rd CDR) was identified as the dominant binding epitope for binding IL-8. Alanine substitutions in this CDR resulted in a 3 to >26 fold decrease in binding affinity. The amino acids, Y597, Y599 and D602 are of particular interest because it was determined from the computer generated model of the anti-IL-8 antibody that these residues are solvent exposed and that these residues might participate in hydrogen bonding or charge interactions with IL-8 or other amino acids of the antibody that influence either binding to IL-8 or the conformation of the CDR-H3

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loop structure. (See the model depicted in Fig. 32). Unexpected increases in binding affinity (1.8 > 2.7 fold) were noted for S528 and S531 of CDR-H1 and S553 of CDR-H2.

Surprisingly, a significant increase in binding affinity was observed in the alanine mutant N35A located in CDR-L1 (light chain, 1st CDR). A 3-6 fold increase in affinity was observed compared to the wild-type h6G4V11 antibody. This augmentation of IL-8 binding could be the result of the close proximity of N35A to CDR-H3. The alanine substitution may have imparted a slight change in the conformation of CDR-L1 which alters the packing interaction of neighboring amino acid residues on CDR-H3, thereby tweaking the loop of CDR-H3 into a conformation that facilitates more appropriate contacts with IL-8. Similarly, N35A may also influence the orientation of amino acids in CDR-L1 or its interaction directly with IL-8. Unexpected increases in affinity (~2 fold) were also observed for S26 of CDR-L1 and H98 of CDR-L3.

J. CHARACTERIZATION OF HUMANIZED ANTI-IL-8 ANTIBODY 6G4V11N35A

Soluble 6G4V11N35A Fab antibody was made by transforming an amber non-suppressor strain of E. coli, 34B8, with pPh6G4.V11 and growing the culture in low phosphate medium for 24 hours. The periplasmic fraction was collected and passed over a Hi-Trap Protein-G column (Pharmacia, Piscataway, NJ.) followed by a desalting and concentration step. The protein was analyzed by SDS-PAGE, mass spectrometry and amino acid analysis. The protein had the correct size and amino acid composition (Fig. 35). The 6G4V11N35A Fab was tested for its ability to inhibit 125 I-IL-8 binding to human neutrophils and to inhibit IL-8 mediated neutrophil chemotaxis as described in Section (B)(1) and (B)(2) above. As shown in Fig. 33, hybridoma-derived intact murine antibody (6G4 murine mAB), recombinant 6G4 murine-human chimera Fab, recombinant humanized Fab versions 1 and 11, and 6G4V11N35A Fab were found to inhibit ¹²⁵I-IL-8 binding to human neutrophils with an average IC₅₀ of 5nM, 8nM, 40nM, 10nM and 3nM, respectively. The 6G4V11N35A Fab had at least a 2-fold higher affinity than the 6G4.2.5 chimera Fab and a 3-fold higher affinity than 6G4V11. As shown in Fig. 34, the 6G4V11N35A Fab was found to inhibit IL-8 mediated neutrophil chemotaxis induced by both wild type and monomeric human IL-8, and by two different animal species of IL-8, namely, rabbit and rhesus. The irrelevant isotype control Fab (4D5) did not inhibit neutrophil migration. The average IC₅₀ values were 3nM (wt IL-8), 1 nM (monomeric IL-8), 5nM (Rabbit IL-8), and 10nM (Rhesus IL-8).

K. CONSTRUCTION OF A 6G4V11N35A F(ab')2 LEUCINE ZIPPER

Production of a F(ab')₂ version of the humanized anti-IL-8 6G4V11N35A Fab was accomplished by constructing a fusion protein with the yeast GCN4 leucine zipper. The expression plasmid p6G4V11N35A.F(ab')₂ was made by digesting the plasmid p6G425chim2.fab2 with the restriction enzymes bsal and apal to remove the DNA sequence encoding the 6G4.2.5 murine-human chimeric Fab and replacing it with a 2620bp bsal-apal fragment from pPh6G4.V11N35A. The plasmid p6G425chim2.fab2 is a derivative of pS1130 which encodes a fusion protein (the GCN4 leucine zipper fused to the heavy chain of

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anti-CD18) and the light chain of anti-CD18 antibody. The expression plasmid p6G4V11N35A.F(ab')₂ was deposited on February 20, 1996 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. 97890. A pepsin cleavage site in the hinge region of the antibody facilitates the removal of the leucine zipper leaving the two immunoglobin monomers joined by the cysteines that generate the interchain disulfide bonds. The DNA and protein sequence of the h6G4V11N35A.F(ab')₂ are depicted in Figs. 35-37.

An expression host cell was obtained by transforming E. coli strain 49D6 with p6G4V11N35A.F(ab')₂ essentially as described in Section (II)(3)(C) above. The transformed host E. coli 49D6 (p6G4V11N35A.F(ab')₂) was deposited on February 20, 1997 at the ATCC and assigned ATCC Accession No. 98332. Transformed host cells were grown in culture, and the 6G4V11N35A F(ab')₂ product was harvested from the host cell periplasmic space essentially as described in Section (II)(3)(F) above.

L. CHARACTERIZATION OF THE HUMANIZED 6G4V11N35A F(ab')2 LEUCINE ZIPPER

The 6G4V11N35A Fab and $F(ab')_2$ were tested for their ability to inhibit ¹²⁵I-IL-8 binding to neutrophils according to the procedures described in Section (B)(1) above. The displacement curves from a representative binding experiment performed in duplicate is depicted in Fig. 38. Scatchard analysis of this data shows that 6G4V11N35A $F(ab')_2$ inhibited ¹²⁵I-IL-8 binding to human neutrophils with an average IC₅₀ of 0.7 nM (+/- 0.2). This is at least a 7 fold increase in affinity compared to the hybridoma-derived intact murine antibody (average IC₅₀ of 5 nM) and at least a 2.8 fold increase in affinity over the Fab version (average IC₅₀ of 2 nM).

The 6G4V11N35A F(ab')2 was also tested for its ability to inhibit IL-8 mediated neutrophil chemotaxis according to the procedures described in Section (B)(2) above. The results of a representative chemotaxis experiment performed in quadruplicate are depicted in Fig. 39. As shown in Fig. 39, the 6G4V11N35A F(ab')₂ inhibited human IL-8 mediated neutrophil chemotaxis. The 6G4V11N35A F(ab')₂ exhibited an average IC50 value of 1.5nM versus 2.7nM for the 6G4V11N35A Fab, which represents an approximately 2 fold improvement in the antibody's ability to neutralize the effects of IL-8. The irrelevant isotype control Fab (4D5) did not inhibit neutrophil migration. Furthermore, the 6G4V11N35A F(ab')2 antibody retained its ability to inhibit IL-8 mediated neutrophil chemotaxis by monomeric IL-8 and by two different animal species of IL-8, namely rabbit and rhesus, in neutrophil chemotaxis experiments conducted as described above. An individual experiment is shown in Fig. 40. The average IC50 values were 1nM IL-8). IL-8), 4nM 2.0nM (Rhesus (monomeric (Rabbit IL-8), and

M. RANDOM MUTAGENESIS OF LIGHT CHAIN AMINO ACID (N35A) IN CDR-L1 OF HUMANIZED ANTIBODY 6G4V11

A 3-fold improvement in the IC₅₀ for inhibiting ¹²⁵I-IL-8 binding to human neutrophils was observed when alanine was substituted for asparagine at position 35 in CDR-L1 (light chain) of the humanized 6G4V11 mAb as described in Section (I) above. This result might be attributed to an improvement in the contact between the antigen-antibody binding interfaces as a consequence of the replacement of a less bulky nonpolar side chain (R-group) that may have altered the conformation of CDR-L1 or neighboring CDR-H3 (heavy chain) to become more accessible for antigen docking. The acceptance of alanine at position 35 of CDR-L1 suggested that this position contributed to improved affinity and that an assessment of the re-modeling of CDR loops / antigen-binding region(s) by other amino acids at this location was warranted. Selection of an affinity matured version of the humanized 6G4.V11 mAB (Kunkel, T. A., <u>Proc. Natl. Acad. Sci. USA</u>, 82:488 (1995)) was accomplished by randomly mutagenizing position 35 of CDR-L1 and constructing an antibody-phage library. The codon for Asparagine (N) at position 35 of CDR-L1, was targeted for randomization to any of the 20 known-amino-acids.

Initially, a stop template, pPh6G4.V11-stop, was made to eliminate contaminating wild-type N35 sequence from the library. This was accomplished by performing site-directed mutagenesis (Muta-Gene Kit, Biorad, Ricmond, CA) of pPH6G4V11 (described in Section (H) above) to replace the codon (AAC) for N35 with a stop codon (TAA) using the primer SL.97.2 (SEQ ID NO:)(Figure 42). The incorporation of the stop codon was confirmed by DNA sequencing. Subsequently, uracil containing single-stranded DNA derived from E. coli CJ236 transformed with the stop template was used to generate an antibodyphage library following the method described by Lowman (Methods in Molecular Biology, 87 Chapter 25: 1-15 (1997). The variants generated from this library were predicted to produce a collection of antibodies containing one of the 20 known amino acids at position N35 in CDR-L1. The amino acid substitutions were accomplished by site-directed mutagenesis using the degenerate oligonucleotide primer (SL.97.3) with the sequence NNS (N = A/G/T/C; S = G/C;) (SEQ ID NO:)(Figure 42). This codon usage should allow for the expression of any of the 20 amino acids - including the amber stop codon (TAG). The collection of antibody-phage variants was transfected into E. coli strain XL-1 blue (Stratagene, San Diego, CA) by electroporation and grown at 37°C overnight to amplify the library. Selection of tight binding humanized 6G4V11 Fab's were accomplished by panning the library on IL-8 coated 96-well plates as described in Section (I) above. Prior to panning, the number of phage/library was normalized to 1.1x10¹³

phage/ml (which produces a maximum OD₂₇₀ reading = 1 OD unit) and IL-8 coated plates were incubated with blocking solution (25mN Carbonate buffer containing 50mg/ml skim milk) for 2 hours before the addition of phage (each sort used eight IL-8 coated wells/library). After the blocking and washing steps, every sort began with the addition of 100ul of antibody-phage (titered at 1.1x10¹³ phage/ml) to each of eight IL-8 coated wells followed by an 1 hour incubation at 25°C. The non-specifically bound antibody-phage were removed by 10 quick washes with PBS-0.05% Tween 20 (PBS-

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Tween). For sort #1, a low stringency wash (100ul PBS-Tween/well for 10 minutes at 25°C) was employed to capture the small proportion of tight binding antibody-phage bound to the immobilized IL-8. The antibody-phage variants specifically bound to IL-8 were eluted with 100ul/well of 200mM Glycine pH 2.0 for 5 minutes at 25°C. The eluted antibody-phage variants from the 8 wells were then pooled and neutralized with 1M Tris-HCl pH 8.0 (1/3 the elution volume). The phage were titered and propagated as described in Section (I) above. The stringency of the washes were successively increased with each round of panning depending upon the percent recovery of phage at the end of a sort. The wash conditions were as follows: sort #2 (4 x 15 minute intervals; total time = 60 minutes) and sort #3 (either #3a: 8 x 15 minute intervals or #3b: 12 x 10 minute intervals; total time = 120 minutes). The total number of phage recovered was progressively reduced after each sort suggesting that non- or weak- binders were being selected against. The recovery of the negative control (the antibody-phage stop variant) was constant throughout the panning (approximately 0.0001 to 0.00001 percent).

Eighteen random variants from sort #3 were analyzed by DNA sequencing to look for an amino acid consensus at position 35 of CDR-L1. The data presented in Figure 43A showed that Glycine occupied position 35 in 33% of the variants sequenced. However, after correcting for the number of NNS codon combinations/amino acid, the frequency of Glycine was reduced to 16.6%. Glutamic Acid was represented with the highest frequency (22%) followed by Aspartic Acid and Glycine (16.6%). The frequencies of recovery of the wild-type Asparagine and substituted Alanine were only 5.6%. Interestingly, the high frequency of Glycine may suggest that a much wider range of conformations might be allowed for the loop of CDR-L1 which may be attributed to the reduction in steric hindrance of bond angle $(\phi-\psi)$ pairing as a result of the single hydrogen atom as the side chain. Conversely, Glutamic Acid at position 35 might restrict the flexibility of the loop by imposing less freedom of rotation imposed by the more rigid and bulky charged polar side chain.

Soluble Fab's of the affinity matured variants (N35G, N35D, N35E and N35A) were made as described in Section (J) above for evaluating their ability to block IL-8 binding. As shown in Figure 43B, variants N35A, N35D, N35E and N35G were found to inhibit ¹²⁵I-IL-8 binding to human neutrophils with an approximate IC₅₀ of 0.2nM, 0.9nM, 0.1nM and 3.0nM, respectively. All of the affinity matured variants showed an improvement in binding IL-8 ranging from 3 - 100 fold compared to the humanized 6G4V11 mAb. The affinity-matured variant, 6G4V11N35E, was 2-fold more potent in blocking IL-8 binding to human neutrophils than the alanine-scan variant, 6G4V11N35A.

Equilibrium and kinetic measurements of variants 6G4V11N35A and 6G4V11N35E were determined using KinEXATM automated immunoassay system (Sapidyne Instruments Inc., Idaho City, ID) as described by Blake *et al.*, J. Biol. Chem. 271: 27677 (1996). The procedure for preparing the antigencoated particles was modified as follows: 1 ml of activated agarose beads (Reacti-Gel 6X; Pierce, Rockford, IL) were coated with antigen in 50mM Carbonate buffer pH 9.6 containing 20ug/ml of human IL-8 and incubated with gentle agitation on a rocker overnight at 25°C. The IL-8 coated beads were then

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washed twice with 1M Tris-HCl pH 7.5 to inactivate any unreactive groups on the beads and blocked with Superblock (Pierce, Rockford, IL) for 1 hour at 25C to reduce non-specific binding. The beads were resuspended in assay buffer (0.1% bovine serum albumin in PBS) to a final volume of 30 ml. A 550ul aliquot of the IL-8 coated bead suspension was used each time to pack a fresh 4mm high column in the KinEXA observation cell. The amount of unbound antibody from the antibody-antigen mixtures captured by the IL-8-coated beads in both the equilibrium and kinetic experiments was quantified using a fluorescently labeled secondary antibody. Murine 6G4.2.5 was detected with a R-PE AffiniPure F(ab')₂ goat anti-mouse IgG, Fc fragment specific 2° antibody (Jackson Immuno Research Laboratories, West Grove, PA) and humanized affinity matured N35A (Fab and F(ab')₂) and N35E Fab were detected with a R-PE AffiniPure F(ab')₂ donkey anti-human IgG (H+L) 2° antibody (Jackson Immunoresearch Laboratories, West Grove, PA); both at a 1:1000 dilution.

Equilibrium measurements were determined by incubating a constant amount of anti-IL-8 antibody (0.005ug/ml) with various concentrations of human IL-8 (0, 0.009, 0.019, 0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5nM). The antibody-antigen mixture was incubated for 2 hours at 25°C to allow the molecules to reach equilibrium. Subsequently, each sample was passed over a naive IL-8 coated bead pack in the KinEXA observation cell at a flow rate of 0.5ml/minute for a total of 9 minutes/sample. The equilibrium constant (Kd) was calculated using the software provided by Sapidyne Instruments Inc.

Rates of association (ka) and dissociation (kd) were determined by incubating together a constant amount of antibody and antigen, and measuring the amount of uncomplexed anti-IL-8 bound to the IL-8 coated beads over time. The concentration of antibody used in the kinetic experiments was identical to that used in the equilibrium experiment described above. Generally, the amount of human IL-8 used was the concentration derived from the binding curves of the equilibrium experiment that resulted in 70% inhibition of anti-IL-8 binding to the IL-8 coated beads. Measurements were made every 15 minutes to collect approximately nine data points. The ka was calculated using the software provided by Sapidyne Instruments, Inc. The off rate was determined using the equation: kd = Kd/ka.

Figure 44 shows the equilibrium constants (Kd) for the affinity matured variants 6G4V11N35E and 6G4V11N35A Fab's were approximately 54pM and 114pM, respectively. The improvement in affinity of 6G4V11N35E Fab for IL-8 can be attributed to a 2-fold faster rate of association (K_{on}) of 4.7x10⁶ for 6G4V11N35E Fab versus 2.0x10⁶ for 6G4V11N35A F(ab')₂. (The Kd of the 6G4V11N35A F(ab')₂ and

6G4V11N35A Fab are similar.) The dissociation rates (K_{off}) were not significantly different. Molecular modeling suggests that substitution of Aspargine with Glutamic Acid might either affect the antibody's interaction with IL-8 directly or indirectly by neutralizing the charge of neighboring residues R98 (CDR-H3) or K50 (CDR-L2) in the CDR's to facilitate contact with IL-8. Another effect might be the formation of a more stable loop conformation for CDR-L1 that could have facilitated more appropriate contacts of other CDR-L1 loop residues with IL-8. The DNA (SEQ ID NO:) and amino acid (SEQ ID NO:)

sequences of p6G4V11N35E.Fab showing the Asparagine to Glutamic Acid substitution in the light chain are presented in Figure 45.

N. CHARACTERIZATION OF HUMANIZED ANTI-IL-8 VARIANT 6G4V11N35E Fab

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The affinity matured Fab variant, 6G4V11N35E, was tested for its ability to inhibit IL-8 mediated neutrophil chemotaxis as described in Section (B)(2) above. The reuseable 96-well chemotaxis chamber described in Section (B)(2) was replaced with endotoxin-free disposable chemotaxis chambers containing 5-micron PVP-free polycarbonate filters (ChemoTx101-5, Neuro Probe, Inc. Cabin John, MD). As illustrated in Figure 46, variant N35E effectively blocks IL-8 mediated neutrophil chemotaxis induced by a 2nM stimulus of either rabbit or human IL-8. In fact, the level of inhibition at antibody concentrations between 3.7nM - 33nM was not significantly different from the buffer control indicating variant N35E could completely inhibit this response. The IC₅₀'s for both rabbit and human IL-8 were approximately 2.8nM and 1.2nM, respectively. The irrelevant isotype control Fab (4D5) did not inhibit neutrophil migation indicating the results observed for the affinity matured variant, N35E, is IL-8 specific.

O. CONSTRUCTION OF HUMANIZED 6G4V11N35E F(ab')2 LEUCINE ZIPPER

A F(ab')₂ expression plasmid for 6G4V11N35E was constructed using methods similar to those described in Section (K) above. The expression plasmid, p6G4V11N35E.F(ab')₂, was made by digesting the plasmid p6G4V11N35A.F(ab')₂ (described in Section (K) above) with the restriction enzymes Apal and Ndel to isolate a 2805 bp fragment encoding the heavy chain constant domain -GCN4 leucine zipper and ligating it to a 3758 bp Apal-Ndel fragment of the pPH6G4V11N35E phage display clone (encoding 6G4V11N35E Fab) obtained as described in Section (M) above. The integrity of the entire coding sequence was confirmed by DNA sequencing.

P. <u>CONSTRUCTION OF THE FULL LENGTH HUMANIZED 6G4V11N35A IgG EXPRESSION</u> PLASMID

The full length IgG₁ version of the humanized anti-IL8 variant 6G4V11N35A was made using a dicistronic DHFR-Intron expression vector (Lucas et al., Nucleic Acids Res.,24: 1774-1779 (1996)) which contained the full length recombinant murine-human chimera of the 6G4.2.5 anti-IL8 mAb. The expression plasmid encoding the humanized variant 6G4V11N35A was assembled as follows. First an intermediate plasmid (pSL-3) was made to shuttle the sequence encoding the variable heavy chain of humanized anti-IL-8 variant 6G4V11N35A to pRK56G4chim.2Vh - which contains the variable heavy region of the chimeric 6G4.5 anti-IL8 antibody. The vector pRK56G4chim.Vh was digested with PvuII and Apal to remove the heavy chain variable region of the chimeric antibody and religated with an 80bp PvuII - XhoI synthetic oligonucleotide (encoding Leu4 to Phe29 of 6G4V11N35A) (Fig. 47) and a 291bp XhoI - ApaI fragment from p6G4V11N35A.7 carrying the remainder of the variable heavy chain sequence f 6G4V11N35A to create pSL-3. This intermediate plasmid was used in conjunction with 2 other plasmids, p6G4V11N35A.F(ab')₂ and p6G425chim2.choSD, to create the mammalian expression plasmid,

p6G4V11N35AchoSD.9 (identified as p6G425V11N35A.choSD in a deposit made on December 16, 1997 with the ATCC and assigned ATCC Accession No. 209552). This expression construct was assembled in a 4-part ligation using the following DNA fragments: a 5,203bp ClaI - BlpI fragment encoding the regulatory elements of the mammalian expression plasmid (p6G425 chim2.choSD), a 451bp ClaI - ApaI fragment containing the heavy chain variable region of the humanized 6G4V11N35A antibody (pSL-3), a 1,921bp ApaI - EcoRV fragment carrying the heavy chain constant region of 6G4V11N35A (p6G425chim2.choSD) and a 554bp EcoRV - BlpI fragment encoding the light chain variable and constant regions of 6G4V11N35A (p6G4V11N35A.F(ab')₂). The DNA sequence (SEQ ID NO:) of clone p6G4V11N35A.choSD.9 was confirmed by DNA sequencing and is presented in Figure 48.

Q. CONSTRUCTION OF THE FULL LENGTH HUMANIZED 6G4V11N35E IgG EXPRESSION PLASMID

A mammalian expression vector for the humanized 6G4V11N35E was made by swapping the light chain variable region of 6G4V11N35A with 6G4V11N35E as follows: a 7,566bp EcoRV - BlpI fragment (void of the 554bp fragment encoding the light chain variable region of 6G4V11N35A) from p6G4V11N35A.choSD.9 was ligated to a 554bp EcoRV - BlpI fragment (encoding the light chain variable region of 6G4V11N35E) from pPH6G4V11N35E.7. The mutation at position N35 of the light chain of p6G4V11N35E.choSD.10 was confirmed by DNA sequencing.

R. STABLE CHO CELL LINES FOR VARIANTS N35A AND N35E

For stable expression of the final humanized IgG1 variants (6G4V11N35A and 6G4V11N35E), Chinese hamster ovary (CHO) DP-12 cells were transfected with the above-described dicistronic vectors (p6G4V11N35A.choSD.9 and p6G4V11N35E.choSD.10, respectively) designed to coexpress both heavy and light chains (Lucas et al., Nucleic Acid Res. 24:1774-79 (1996)). Plasmids were introduced into CHO DP12 cells via lipofection and selected for growth in GHT-free medium (Chisholm, V. High efficiency gene transfer in mammalian cells. In: Glover, DM, Hames, BD. DNA Cloning 4. Mammalian systems. Oxford Univ. Press, Oxford pp 1-41 (1996)). Approximately 20 unamplified clones were randomly chosen and reseeded into 96 well plates. Relative specific productivity of each colony was monitored using an ELISA to quantitate the full length human IgG accumulated in each well after 3 days and a fluorescent dye, Calcien AM, as a surrogate marker of viable cell number per well. Based on these data, several unamplified clones were chosen for further amplification in the presence of increasing concentrations of methotrexate. Individual clones surviving at 10, 50, and 100 nM methotrexate were chosen and transferred to 96 well plates for productivity screening. One clone for each antibody (clone#1933 alL8.92 NB 28605/12 f r 6G4V11N35A; clone#1934 aIL8.42 NB 28605/14 for 6G4V11N35E), which reproducibly exhibited high specific productivity, was expanded in T-flasks and used to inoculate a spinner culture. After several passages, the suspension-adapted cells were used to inoculate production cultures in GHT-containing, serum-free media supplemented with various hormones and protein hydrolysates. Harvested cell culture fluid containing recombinant humanized anti-IL8 was purified using protein A-Sepharose CL-4B. The purity after this step was approximately 99%. Subsequent purification to homogeneity was carried out

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using an ion exchange chromatography step. Production titer of the humanized 6G4V11N35E IgG1 antibody after the first round of amplification and 6G4V11N35A IgG1 after the second round of amplification were 250mg/L and 150mg/L, respectively.

S. CHARACTERIZATION OF THE HUMANIZED 6G4V11N35A/E IgG VARIANTS

The humanized full length IgG variants of 6G4.2.5 were tested for their ability to inhibit 125 I-IL-8 binding and to neutralize activation of human neutrophils; the procedures are described in Sections (B)(1) and (B)(2) above. As shown in Figure 49, the full length IgG1 forms of variants 6G4V11N35A and 6G4V11N35E equally inhibited 125 I-IL-8 binding to human neutrophils with approximate IC₅₀'s of 0.3nM and 0.5nM, respectively. This represents a 15 - 25 fold improvement in blocking binding of IL-8 compared to the full length murine mAb (IC₅₀ = 7.5nM). Similarly, the two anti-IL-8 variants showed equivalent neutralizing capabilities with respect to inhibiting IL-8 mediated human neutrophil chemotaxis (Figures 50A-50B). The IC₅₀'s of 6G4V11N35A IgG1 and 6G4V11N35E IgG1 for human IL-8 were 4.0nM and 6.0nM, respectively, and for rabbit IL-8 were 4.0nM and 2.0nM, respectively. The irrelevant isotype control Fab (4D5) did not inhibit neutrophil migration.

The affinity for IL-8 of these variants relative to the murine 6G4.2.5 mAb was determined using KinExA as described in Section (M). Figure 51 shows the equilibrium constant (Kd) for the full length affinity matured variants 6G4V11N35E IgG1 and 6G4V11N35A IgG1 were approximately 49pM and 88pM, respectively. The Kd for 6G4V11N35A IgG1 was determined directly from the kinetic experiment. As reported with their respective Fabs, this improvement in affinity might be attributed to an approximate 2-fold increase in the on-rate of 6G4V11N35E IgG1 (ka = 3.0x10⁶) compared to that of 6G4V11N35A IgG1 (ka = 8.7x10⁵). In addition, these results were confirmed by a competition radio-immune assay using iodinated human IL-8. 50pM of 6G4V11N35A IgG1 or 6G4V11N35E IgG1 was incubated for 2 hours at 25°C with 30-50pM of ¹²⁵I-IL-8 and varying concentrations (0 to 100nM) of unlabeled IL-8. The antibody-antigen mixture was then incubated for 1 hour at 4C with 10ul of a 70% slurry of Protein-A beads (pre-blocked with 0.1% BSA). The beads were briefly spun in a microcentrifuge and the supernatant discarded to remove the unbound ¹²⁵I-IL-8. The amount of ¹²⁵I-IL-8 specifically bound to the anti-IL-8 antibodies was determined by counting the protein-A pellets in a gamma counter. The approximate Kd values were similar to those determined by KinEXA. The average Kd for 6G4V11N35A IgG1 and 6G4V11N35E IgG1 were 54pM (18 -90pM) and 19pM (5-34pM), respectively (Figure 52).

T. CONSTRUCTION OF HUMANIZED 6G4V11N35A/E Fab's FOR MODIFICATION BY POLYETHYLENE GLYCOL

A Fab' expression vector for 6G4V11N35A was constructed by digesting p6G4V11N35A.F(ab')₂ with the restriction enzymes Apal and Ndel to remove the 2805 bp fragment encoding the human IgG₁

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constant domain fused with the yeast GCN4 leucine zipper and replacing it with the 2683bp Apal-NdeI fragment from the plasmid pCDNA.18 described in Eigenbrot et al., Proteins: Struct. Funct. Genet., 18: 49-62 (1994). The pCDNA.18 Apal-NdeI fragment carries the coding sequence for the human constant IgG1 heavy domain, including the free cysteine in the hinge region that was used to attach the PEG molecule. The 3758bp Apal-NdeI fragment (encodes the light chain and heavy variable domain of 6G4V11N35A) isolated from p6G4V11N35A.F(ab')₂ was ligated to the 2683bp Apal-NdeI fragment of pCDNA.18 to create p6G4V11N35A.PEG-1. The integrity of the entire coding sequence was confirmed by DNA sequencing. The nucleotide and translated amino acid sequences of heavy chain constant domain with the cysteine in the hinge are presented in Figure 53.

A Fab' expression plasmid for 6G4V11N35E was made similarly by digesting pPH6G4V11N35E (from Section (O) above) with the restriction enzymes Apal and NdeI to isolate the 3758bp Apal-NdeI DNA fragment carrying the intact light chain and heavy variable domain of 6G4V11N35E and ligating it to the 2683 bp Apal-NdeI DNA fragment from p6G4V11N35A.PEG-1 to create p6G4V11N35E.PEG-3. The integrity of the entire coding sequence was confirmed by DNA sequencing.

Anti-IL-8 6G4V11N35A Fab' variant was modified with 20 kD linear methoxy-PEG-maleimide, 30 kD linear methoxy-PEG-maleimide, 40 kD linear methoxy-PEG-maleimide, or 40 kD branched methoxy-PEG-maleimide as described below. All PEG's used were obtained commercially from Shearwater Polymers, Inc.

a. MATERIALS AND METHODS

Fab'-SH Purification

A Fab'-SH antibody fragment of the affinity matured antibody 6G4V11N35A was expressed in E. coli grown to high cell density in the fermentor as described by Carter et al., Bio/Technology 10, 163-167 (1992). Preparation of Fab'-SH fragments was accomplished by protecting the Fab'-SH fragments with 4',4'-dithiodipyridine (PDS), partially purifying the protected Fab'-PDS fragments, deprotect the Fab'-PDS with dithiothreitol (DTT) and finally isolate the free Fab'-SH by using gel permeation chromatography.

Protection of Fab'-SH with PDS

Fermentation paste samples were dissolved in 3 volumes of 20mM MES, 5mM EDTA, pH 6.0 containing 10.7mg of 4',4'-dithiodipyridine per gram fermentation paste, resulting in a suspension with a pH close to 6.0 The suspension was passed through a homogenizer followed by addition of 5% PEI (w/v), pH 6 to the homogenate to a final concentration of 0.25%. The mixture was then centrifuged to remove solids and the clear supernatant was conditioned to a conductivity of less than 3mS by the addition of cold water.

Partial purification of the Fab'-SH molecule using ion exchange chromatography

The conditioned supernatant was loaded onto an ABX (Baker) column equilibrated in 20 mM MES, pH 6.0. The column was washed with the equilibration buffer followed by elution of the Fab'-SH with a 15 column volume linear gradient from 20 mM MES, pH 6.0 to 20 mM MES, 350 mM sodium chloride. The column was monitored by absorbance at 280nm, and the eluate was collected in fracti ns.

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Deprotection of the Fab'-SH antibody fragments with DTT

The pH of the ABX pool was adjusted to 4.0 by the addition of dilute HCl. The pH adjusted solution was then deprotected by adding DTT to a final concentration of 0.2mM. The solution was incubated for about 30 minutes and then applied to a gel filtration Sephadex G25 column, equilibrated with 15mM sodium phosphate, 25mM MES, pH 4.0. After elution, the pH of the pool was raised to pH 5.5 and immediately flash frozen at -70°C for storage or derivatized with PEG-MAL as described below.

Alternative Fab'-SH Purification

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Alternatively Fab'-SH fragments can be purified using the following procedure. 100 g fermentation paste is thawed in the presence of 200 ml 50 mM acetic acid, pH 2.8, 2 mM EDTA, 1 mM PMSF. After mixing vigorously for 30 min at room temperature, the extract is incubated with 100 mg hen egg white lysozyme. DEAE fast flow resin (approximately 100 mL) is equilibrated with 10 mM MES, pH 5.5, 1 mM EDTA on a sintered glass funnel. The osmotic shock extract containing the Fab'-SH fragment is then filtered through the resin.

A protein G Sepharose column is equilibrated with 10 mM MES, pH 5.5, 1 mM EDTA and then loaded with the DEAE flow-through sample. The column is washed followed by three 4 column volume washes with 10 mM MES, pH 5.5, 1 mM EDTA. The Fab'-SH antibody fragment containing a free thiol is eluted from the column with 100 mM acetic acid, pH 2.8, 1 mM EDTA. After elution, the pH of the pool is raised to pH 5.5 and immediately flash frozen at -70°C for storage or derivatized with PEG-MAL as described below.

Preparation of Fab'-S-PEG

The free thiol content of the Fab'-SH preparation obtained as described above was determined by reaction with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) analysis according to the method of Creighton in Protein Structure: A Practical Approach, Creighton, T.E., ed, IRL Press (Oxford, UK: 1990), pp. 155-167. The concentration of free thiol was calculated from the increase on absorbance at 412 nm, using $e_{412} = 14,150 \text{ cm}^{-1} \text{ M}^{-1}$ for the thionitrobenzoate anion and a $M_r = 48,690$ and $e_{280} = 1.5$ for the Fab'-SH antibody. To the Fab'-SH protein G Sepharose pool, or the deprotected Fab'-SH gel permeation pool, 5 molar equivalents of PEG-MAL were added and the pH was immediately adjusted to pH 6.5 with 10% NaOH.

The Fab'-S-PEG was purified using a 2.5 x 20 cm cation exchange column (Poros 50-HS). The column was equilibrated with a buffer containing 20 mM MES, pH 5.5. The coupling reaction containing the PEGylated antibody fragment was diluted with deionized water to a conductivity of approximately 2.0 mS. The conditioned coupling reaction was then loaded onto the equilibrated Poros 50 HS column. Unreacted PEG-MAL was washed from the column with 2 column volumes of 20 mM MES, pH 5.5. The Fab'-S-PEG was eluted from the column using a linear gradient from 0 to 400 mM NaCl, in 20 mM MES pH 5.5, over 15 column volumes.

Alternatively a Bakerbond ABX column can be used to purify the Fab'-S-PEG molecule. The column is equilibrated with 20 mM MES, pH 6.0 (Buffer A). The coupling reaction is diluted with deionized water until the conductivity equaled that of the Buffer A (approximately 2.0 mS) and loaded onto the column. Unreacted PEG-MAL is washed from the column with 2 column volumes of 20 mM MES, pH 6.0. The Fab'-S-PEG is eluted from the column using a linear gradient from 0 to 100 mM (NH₄)₂SO₄, in 20 mM MES pH 6.0, over 15 column volumes.

Size Exclusion Chromatography

The hydrodynamic or effective size of each molecule was determined using a Pharmacia Superose-6 HR 10/30 column (10x300mm). The mobile phase was 200 mM NaCl, 50 mM sodium phosphate pH 6.0. Flow rate was at 0.5 ml/min and the column was kept at ambient temperature. Absorbance at 280 nm was monitored where PEG contributed little signal. Biorad MW standards containing cyanocobalamin, myoglobin, ovalbumin, IgG, Thyroglobulin monomer and dimer were used to generate a standard curve from which the effective size of the pegylated species was estimated.

b. RESULTS

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Size Exclusion Chromatography

The effective size of each modified species was characterized using size exclusion chromatography. The results are shown in Fig. 60 below. The theoretical molecular weight of the anti-IL8 Fab fragments modified with PEG 5kD, 10kD, 20kD, 30kD, 40kD (linear), 40kD (branched) or 100,000kD is shown along with the apparent molecular weight of the PEGylated fragments obtained by HPLC size exclusion chromatography. When compared to the theoretical molecular weight of the Fab'-S-PEG fragments, the apparent molecular weight (calculated by size exclusion HPLC) increases dramatically by increasing the size of the PEG attached to the fragments. Attachment of a small molecular weight PEG, for example PEG 10,000D only increases the theoretical molecular weight of the PEGylated antibody fragment (59,700 D) by 3 fold to an apparent molecular weight of 180,000D. In contrast attachment of a larger molecular weight PEG for example 100,000D PEG to the antibody fragment increases the theoretical molecular weight of the PEGylated antibody fragment (158,700 D) by 12 fold to an apparent molecular weight of 2,000,000D.

SDS-PAGE

In Fig. 61, the upper panel shows the size of the anti-IL-8 Fab fragments modified with PEG of molecular weight 5kD (linear), 10kD (linear), 20kD (linear), 30kD (linear), 40kD (linear), 40kD (branched) or 100kD (linear) under reduced conditions. The unmodified Fab is shown in lane 2 from right to left. Both the heavy and light chains of the Fab had a molecular weight of approximately 30kD as determined by PAGE. Each PEGylated fragment sample produced two bands: (1) a first band (attributed to the light chain) exhibiting a molecular weight of 30kD; and (2) a second band (attributed to the heavy chain to which the PEG is attached specifically at the hinge SH) exhibiting increasing molecular weights of 40, 45, 70, 110, 125, 150 and 300kD. This result suggested that PEGylation was specifically restricted to the heavy chain of the Fab's whereas the light chain remained unmodified.

The lower panel is non-reduced PAGE showing the size of the anti-IL-8 Fab fragments modified with PEG of molecular weight 5kD (linear), 20kD (linear), 30kD (linear), 40kD (linear), 40kD (branched), or 100kD (linear). The PEGylated fragments exhibited molecular weights of approximately 70kD, 115kD, 120kD, 140kD, 200kD and 300kD.

The SDS PAGE gels confirm that all Fab'-S-PEG molecules were purified to homogeneity and that the molecules differed only with respect to the size of the PEG molecule attached to them.

U. AMINE SPECIFIC PEGYLATION OF ANTI-IL-8 F(ab')₂ FRAGMENTS

Pegylated F(ab')₂ species were generated by using large MW or branched PEGs in order to achieve a large effective size with minimal protein modification which might affect activity. Modification involved N-hydroxysuccinamide chemistry which reacts with primary amines (lysines and the N-terminus). To decrease the probability of modifying the N-terminus, which is in close proximity to the CDR region, a reaction pH of 8, rather than the commonly used pH of 7, was employed. At pH 8.0, the amount of the reactive species (charged NH₃⁺) would be considerably more for the ε-NH2 group of lysines (pK_a=10.3)

than for the α-NH2 group (pK_a of approximately 7) of the amino-terminus. For the linear PEGs, a methoxy-succinimidyl derivative of an NHS-PEG was used because of the significantly longer half-life in solution (17 minutes at 25°C at pH 8.0) compared to the NHS esters of PEGs (which have 5-7 minute half life under the above conditions). By using a PEG that is less prone to hydrolysis, a greater extent of modification is achieved with less PEG. Branched PEGs were used to induce a large increase in effective size of the antibody fragments.

a. MATERIALS

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All PEG reagents were purchased from Shearwater Polymers and stored at -70°C in a desiccator: branched N-hydroxysuccinamide-PEG (PEG2-NHS-40KDa) has a 20 kDa PEG on each of the two branches, methoxy-succinimidyl-propionic acid-PEG (M-SPA-20000) is a linear PEG molecule with 20 kDa PEG. Protein was recombinantly produced in *E. coli* and purified as a (Fab)'₂ as described in Sections (K) and (O) above.

b. METHODS

IEX method: A J. T. Baker Wide-Pore Carboxy-sulfone (CSX), 5 micron, 7.75 x 100 mm HPLC column was used for fractionation of the different pegylated products, taking advantage of the difference in charge as the lysines are modified. The column was heated at 40°C. A gradient as shown in Table 7 below was used where Buffer A was 25 mM sodium Borate/25 mM sodium phosphate pH 6.0, and Buffer B was 1 5.0. ammonium sulfate, and Buffer C was 50 mM sodium acetate Ħσ

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mass accuracy of ~5%.

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5	Time (min)	%B	%C	flow mL/min
	0	10	10	1.5
	20	18	7.5	1.5
	25	25	7.5	1.5
10	27	70	3.0	2.5
	29	70	3.0	2.5
	30	10	10	2.5
	33	10	10	2.5

SEC-HPLC: The hydrodynamic or effective size of each molecule was determined using a Pharmacia Superose-6 HR 10/30 column (10x300mm). The mobile phase was 200 mM NaCl, 50 mM sodium phosphate pH 6.0. Flow rate was at 0.5 ml/min and the column was kept at ambient temperature. Absorbance at 280 nm was monitored where PEG contributed little signal. Biorad MW standards containing cyanocobalamin, myoglobin, ovalbumin, IgG, Thyroglobulin monomer and dimer were used to generate a standard curve from which the effective size of the pegylated species was estimated.

SEC-HPLC-Light Scattering: For determination of the exact molecular weight, this column was connected to an on-line light scattering detector (Wyatt Minidawn) equipped with three detection angles of 50°, 90°, and 135° C. A refractive index detector (Wyatt) was also placed on-line to determine concentration. All buffers were filtered with Millipore 0.1 μ filters; in addition al 0.02 μ Whatman Anodisc 47 was placed on-line prior to the column.

The intensity of scattered light is directly proportional to the molecular weight (M) of the scattering species, independent of shape, according to:

$$M = R_0/K \cdot c$$

where R_0 is the Rayleigh ratio, K is an optical constant relating to the refractive index of the solvent, the wavelength of the incident light, and dn/dc, the differential refractive index between the solvent and the solute with respect to the change in solute concentration, c. The system was calibrated with toluene (R_0 of 1.406×10^{-5} at 632.8 nm); a dn/dc of 0.18, and an extinction coefficient of 1.2 was used. The system had a

SDS-PAGE: 4-12% Tris-Glycine Novex minigels were used along with the Novex supplied Tris-Glycine running buffers. 10-20 ug of protein was applied in each well and the gels were run in a cold box at 150 mV/gel for 45 minutes. Gels were then stained with colloidal Coomassie Blue (Novex) and then washed with water for a few hours and then preserved and dried in drying buffer (Novex)

Preparation of a linear(1)20KDa-(N)-(Fab')2: A 4 mg/ml solution of anti-IL8 formulated initially in a pH 5.5 buffer was dialyzed overnight against a pH 8.0 sodium phosphate buffer. 5 mL protein

was mixed at a molar ratio of 3:1. The reaction was carried out in a 15mL polypropylene Falcon tube and the PEG-was-added while vortexing the sample at low speed for 5 seconds. It was then placed on a nutator for 30 minutes. The extent of modification was evaluated by SDS-PAGE. The whole 5 ml reaction mixture was injected on the IEX for removal of any unreacted PEG and purification of singly or doubly pegylated species. The above reaction generated a mixture of 50% singly-labeled anti-IL8. The other 50% unreacted anti-IL8 was recycled through the pegylation/purification steps. The pooled pegylated product was dialyzed against a pH 5.5 buffer for in vitro assays and animal PK studies. Endotoxin levels were measured before administration to animals or for the cell based assays. Levels were below 0.5 eu/ml. The fractions were also run on SDS-PAGE to confirm homogeneity. Concentration of the final product was assessed by absorbance at 280 nm using an extinction coefficient of 1.34, as well as by amino acid analysis.

Preparation of a branched(1)40KDa-(N)-(Fab')2: A 4 mg/mL solution of anti-IL8 (Fab')₂ formulated in a pH 5.5 buffer was dialyzed overnight against a pH 8.0 phosphate buffer. Solid PEG powder was added to 5 mL protein in two aliquots to give a final PEG:protein molar ratio of 6:1. Each solid PEG aliquot was added to the protein in a 15 mL polypropylene Falcon tube while vortexing at low speed for 5 sec, and then placing the sample on a nutator for 15 minutes. The extent of modification was evaluated by SDS-PAGE using a 4-12% Tris-Glycine (Novex) gel and stained with colloidal Coomasie blue (Novex). The 5 mL PEG-protein mixture was injected on the ion exchange column for removal of any unreacted PEG. The above reaction generated a mixture of unreacted (37%), singly-labelled (45%), doubly and triply-labeled (18%) species. These were the optimal conditions for obtaining the greatest recovery of the protein with only 1 PEG per antibody rather than the higher molecular weight adducts. The unmodified anti-IL8 was recycled. The pegylated products were separated and fractionated in falcon tubes and then dialyzed against a pH 5.5 buffer for assays and animal PK studies. Endotoxin levels were below 0.5 eu/ml. The fractions were also run on SDS-PAGE to confirm homogeneity. The concentration of the final product was assessed by absorbance at 280 nm using an extinction coefficient of 1.34, as well as by amino acid analysis.

Preparation of branched(2)-40KDa-(N)(Fab')2: This molecule was most efficiently made by adding three times in 15 minute intervals a 3:1 molar ratio of PEG to the already modified branched(1)-40KDa-(N)-(Fab')2. The molecule was purified on IEX as 50% branched(2)-40KDa-(N)-(Fab')2. The unmodified molecule was recycled until ~20 mg protein was isolated for animal PK studies. The product was characterized by SEC-light scattering and SDS-PAGE.

c. RESULTS

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PEGs increased the hydrodynamic or effective size of the product significantly as determined by gel filtration (SEC-HPLC). Figure 62 shows the SEC profile of the pegylated F(ab')₂ species with UV detection at 280 nm. The hydrodynamic size of each molecule was estimated by reference to the standard MW calibrators. As summarized in Figure 62, the increase in the effective size of (Fab')₂ was about 7-fold

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by adding one linear 20 kDa PEG molecule and about 11-fold by adding one branched ("Br(1)") 40 kDa PEG molecule, and somewhat more with addition of two branched ("Br(2)") PEG molecules.

Light scattering detection gave the exact molecular weight of the products and confirmed the extent of modification (Figure 63). The homogeneity of the purified material was shown by SDS-PAGE (Figure 64). Underivatized F(ab')₂ migrated as a 120 kDa species, the linear(1)20KD-(N)-F(ab')₂ migrated as a band at 220kDa, the Br(1)-40KD(N)-F(ab')₂ migrated as one major band at 400 kDa, and the Br(2)-40KD-(N)-F(ab')₂ migrated as a major band at around 500 kDa. The proteins appeared somewhat larger than their absolute MW due to the steric effect of PEG.

V. <u>IN VITRO ACTIVITY CHARACTERIZATION OF PEG MODIFIED Fab' FRAGMENTS OF</u> 6G4V11N35A (MALEIMIDE CHEMICAL COUPLING METHOD)

Anti-IL-8 6G4V11N35A Fab' variants modified with 5-40kD linear PEG molecules and a 40kD branched PEG molecule were tested for their ability to inhibit both IL-8 binding and activation of human neutrophils; the procedures were described in Sections (B)(1), (B)(2) and (B)(3) above. The binding curves and IC₅₀'s for PEG-maleimide modified 6G4V11N35A Fab' molecules are presented in Figures 54A-54C. The IC₅₀ of the 5kD pegylated Fab' (350pM) and the average IC₅₀ of the Fab control (366pM) were not significantly different, suggesting that the addition of a 5kD MW PEG did not affect the binding of IL-8 to the modified Fab' (Figure 54A). However, a decrease in the binding of IL-8 to the 10kD and 20kD pegylated Fab' molecules was observed as depicted by the progressively higher IC₅₀'s (537pM and 732pM, respectively) compared to the average IC₅₀ of the native Fab. These values represent only a minimal loss of binding activity (between 1.5- and 2.0-fold). A less pronounced difference in IL-8 binding was observed for the 30kD and 40kD linear PEG antibodies (Figure 54B). The IC₅₀'s were 624pM and 1.1nM, respectively, compared to the 802pM value of the Fab control. The 40kD branched PEG Fab' showed the largest decrease in IL-8 binding (2.5 fold) relative to the native Fab (Figure 54C). Nevertheless, the reduction in binding of IL-8 by these pegylated Fab's is minimal.

The ability of the pegylated antibodies to block IL-8 mediated activation of human neutrophils was demonstrated using the PMN chemotaxis (according to the method described in Section B(2) above) and β-glucuronidase release (according to the method described in Lowman et al., J. Biol. Chem., 271: 14344 (1996)) assays. The IC₅₀'s for blocking IL-8 mediated chemotaxis are shown in Figures 55A-55C. The 5-20kD linear pegylated Fab' antibodies were able to block IL-8 mediated chemotaxis within 2-3 fold of the unpegylated Fab control (Figure 55A). This difference is not significant because the inherent variation can be up to 2 fold for this type of assay. However, a significant difference was detected for the 30kD and 40kD linear pegylated Fab' antibodies as illustrated by the higher IC₅₀'s of the 30kD linear PEG-Fab' (2.5nM) and 40kD linear PEG-Fab' (3.7nM) compared to the Fab control (0.8nM) (Figure 55B).

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The ability of the 40kD branched PEG Fab' molecule to block IL-8 mediated chemotaxis was similar to that of the 40kD linear PEG Fab' (Figure 55C). At most, the ability of the pegylated Fab' antibodies to block IL-8 mediated chemotaxis was only reduced 2-3 fold. Furthermore, release of β-glucuronidase from the granules of neutrophils was used as another criteria for assessing IL-8 mediated activation of human PMNs. Figure 56A (depicting results obtained with 5 kD, 10 kD and 20 kD linear PEGs), Figure 56B (depicting results obtained with 30 kD and 40 kD linear PEGs), and Figure 56C (depicting results obtained with 40 kD branched PEG) show that all the pegylated Fab' antibodies were able to inhibit IL-8 mediated release of β-glucuronidase as well as or better than the unpegylated Fab control. The data collectively shows that the pegylated Fab' variants are biological active and are capable of inhibiting high amounts of exogenous IL-8 in in-vitro assays using human neutrophils.

W. <u>IN VITRO ACTIVITY CHARACTERIZATION OF PEG MODIFIED F(ab')</u> FRAGMENTS OF 6G4V11N35A (SUCCINIMIDYL CHEMICAL COUPLING METHOD)

The anti-IL-8 variant 6G4V11N35A F(ab')₂ modified with (a) a single 20kD linear PEG molecule per F(ab')₂, (b) a single 40kD branched PEG molecule per F(ab')₂, (c) with three, four, or five 20 kD linear PEG molecules per F(ab')₂; (2) species having four 20 kD linear PEG molecules per F(ab')₂; (2) species having four 20 kD linear PEG molecules per F(ab')₂; and (3) species having five 20 kD linear PEG molecules per F(ab')₂; denoted as "20 kD linear PEG (3,4,5) F(ab')₂"), or (d) with two 40kD branched PEG molecules per F(ab')₂ (denoted as "40 kD branch PEG (2) F(ab')₂"), were tested for their ability to inhibit ¹²⁵I-IL-8 binding and to neutralize activation of human neutrophils. The procedures used are described in Sections (B)(1), (B)(2) and (B)(3) above. The binding curves for pegylated F(ab')₂ variants are shown in Figures 57A-57B. No significant differences were observed amongst the F(ab')₂ control, the single 20kD linear PEG-modified F(ab')₂, and the single 40kD branched PEG-modified F(ab')₂ (Figure 57A). However, the F(ab')₂ variants containing multiple PEG molecules showed a slight reduction (less than 2-fold) in their ability to bind IL-8. The IC₅₀'s of the 20kD linear PEG (3,4,5) F(ab')₂ and 40kD branch PEG (2) F(ab')₂ variants were 437pM and 510pM, respectively, compared to 349pM of the F(ab')₂ control (Figure 57B).

The ability of these pegylated $F(ab')_2$ variants to block IL-8 mediated neutrophil chemotaxis is presented in Figures 58A-58B. Consistent with the PMN binding data, the single linear and branched PEG $F(ab')_2$ variants were able to block IL-8 mediated chemotaxis similar to the unpegylated $F(ab')_2$ control (Figure 58A). The ability of the 40kD branch PEG (2) $F(ab')_2$ variant to inhibit PMN chemotaxis was

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identical to the control F(ab')₂ while the 20kD linear PEG (3,4,5) F(ab')₂ mixture was able to inhibit within 3-fold of the control antibody (Figure 58B).

Shown in Figures 59A and 59B are the results of the β -glucuronidase release assay which is a measure of degranulation by IL-8 stimulated human neutrophils. The single 20kD linear PEG-modified $F(ab')_2$ and the single 40kD branched PEG-modified $F(ab')_2$ variants were able to inhibit release of β -glucuronidase as well as the $F(ab')_2$ control (Figure 59A). The 40kD branch PEG (2) $F(ab')_2$ inhibited this response within 2-fold of the $F(ab')_2$ control (Figure 59B). The 20kD linear PEG (3,4,5) molecule was not tested. Overall, the $F(ab')_2$ pegylated anti-IL-8 antibodies were biologically active and effectively prevented IL-8 binding to human neutrophils and the signaling events leading to cellular activation.

10 X. PHARMACOKINETIC AND SAFETY STUDY OF EIGHT CONSTRUCTS OF PEGYLATED ANTI-IL-8 (HUMANIZED) F(AB')2 AND FAB' FRAGMENTS IN NORMAL RABBITS FOLLOWING INTRAVENOUS ADMINISTRATION

The objective of this study was to evaluate the effect of pegylation on the pharmacokinetics and safety of six pegylated humanized anti-IL-8 constructs (pegylated 6G4V11N35A.Fab' and pegylated 6G4V11N35A.F(ab')₂ obtained as described in Sections (T) and (U) above) relative to the non-pegylated fragments in normal rabbits. Eight groups of two/three male rabbits received equivalent protein amounts of pegylated 6G4V11N35A.Fab' or pegylated 6G4V11N35A.F(ab')₂ constructs (2 mg/kg) via a single intravenous (IV) bolus dose of one anti-IL8 construct. Serum samples were collected according to the schedule shown in Table 8 below and analyzed for anti-IL8 protein concentrations and antibody formation against anti-IL8 constructs by ELISA.

Table 8

Group No.	Dose level/ Route	Material	Blood Collection
1		Fab' control	0,5,30 min; 1,2,3,4,6,8,10, 14,20,24,360 hr
2		linear(1)20K(s)Fab'	
3	ŕ	linear(1)40K(s)Fab'	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,72,96,168,216,
4	2 mg/kg	branched(1)40K(N)F(ab')2	264,336,360 hr
5	(protein conc.) IV bolus	F(ab') ₂ control	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,52,56,336 hr

Group No.	Dose level/ Route	. Material	Blood Collection
6		branched(2)40K(s)Fab'	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,72,96,168,216,264,3 36 hr; Day 17,21, 25
7		branched(2)40K(N)F(ab') ₂	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,72,144,192, 240 hr; Day 13, 16, 20, 23
. 8		linear(1)30K(s)Fab'	0,5,30 min; 1,2,4,6,8,10,12, 24,28,32,48,72,96,168,216,264,3 36 hr; Day 17,21, 25

a. METHODS

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Three male New Zealand White (NZW) rabbits per group (with exception to Group 7, n=2) received an equivalent amount of 6G4V11N35A protein (Fab' or F(ab')₂) construct at 2 mg/kg via an IV bolus dose in a marginal ear vein. Amino acid composition analysis and absorbance at 280 nm using extinction coefficients of 1.26 for 6G4V11N35A Fab' constructs and 1.34 for 6G4V11N35A F(ab')₂ constructs were performed to determine the protein concentration. Whole blood samples were collected via an ear artery cannulation (ear opposing dosing ear) at the above time points. Samples were harvested for serum and assayed for free 6G4V11N35A Fab' or F(ab')₂ constructs using an IL-8 Binding ELISA. Assays were conducted throughout the study as samples became available. All animals were sacrificed following the last blood draw, and necropsies were performed on all animals in Groups 1, 4–8. Due to the development of antibodies against the 6G4V11N35A constructs, non-compartmental pharmacokinetic analysis was conducted on concentration versus time data only up to 168 hours.

b. RESULTS

In four animals (Animals B, P, Q, V), interference to rabbit serum in the ELISA assay was detected (i.e. measurable concentrations of anti-IL8 antibodies at pre-dose). However, because these values were at insignificant levels and did not effect the pharmacokinetic analysis, the data were not corrected for this interference.

One animal (Animal G; Group 3) was exsanguinated before the termination of the study and was excluded from the pharmacokinetic analysis. At 4 hours, the animal showed signs of a stroke that was not believed to be drug related, as this can occur in rabbits following blood draws via ear artery cannulation.

The mean concentration-time profiles of the eight anti-IL8 constructs in normal rabbits are depicted in Fig. 65, and the pharmacokinetic parameters for the eight constructs are summarized in Table 9 below. Significant antibodies to the anti-IL-8 constructs were present at Day 13/14 in all dose groups except Group 1 (Fab' control).

Table 9. Pharmacokinetic parameters.

Molecule			Fab'		-F(ab') ₂			
Group No.	1	2	8	3	6	5	4	7
PEG structure		linear	linear	linear	branched	_ 1	branched	branched
Number of PEGs	_	1	1	1	1	_	1	2
PEG MW	_	20K	30K	40K	40K		40K	40K
Dose (mg/kg)	2	2	2	2	2	2	2	2
V _c (mL/kg) ^a	58±3	36±3	35±1	34	44±1	45±5	36±1	. 32
V _{ss} (mL/kg)	68±8	80±8	110±15	79	88±21	59±4	50±3	52
c Cmax (μg/mL)	35±1	58±3	57±1	60	45±1	45±6	56±2	62
d Tmax (min)	5	5	5	5	5	5	5	5
t _{1/2} term (hr)	3.0±0.9	44±2	43±7	50	105±11	8.5±2.1	45±3	48
f AUC ₀ . (hr•μg/mL)	18±3	80±74_	910±140	1600	3400±1300	140±3	2200±77	2500
CL (mL/hr/kg)	110±17	2.5±0.2	2.2±0.4	1.3	0.63±0.20	14±0	0.92±0.03	0.83
MRT (hr) h	0.61±0.15	32±2	45±9	63	140±18	4.2±0.3	55±3	64
No. of Animals	3	3	3	2	3	3	3	2

Initial volume of distribution.

The initial volume of distribution approximated the plasma volume for both the Fab' and F(ab')2.

Pegylation decreased serum CL of anti-IL8 fragments and extended both the terminal half-life and MRT as shown in Table 10 below.

Table 10. Fold decrease/increase in clearance, terminal half-life & MRT of pegylated anti-IL8 fragments.

anti-IL8 fra	gment	Fab'	Fab' F(ab') ₂										
Group No.		11	2	8	3	6	5	4	7				
PEG structi	ıre		linear	linear	linear	bran.	1-	bran.	bran.				
No. of PEG		 _	1	1	1	1	 -	1	2				
PEG MW		-	20K	30K	40K	40K		40K	40K				
CL:	mean (mL/hr/kg)	110	2.5	2.2	1.3	0.63	14	0.92	0.83				
·	fold decrease	1	46	51	90	180	1	15	17				
1/2 term :	mean (hr)	3.0	44	43	50	110	8.5	45	48				
	fold increase	1	14	14	17	35	1	5.3	5.7				
MRT:	mean (hr)	0.61	32	45	63	140	4.2	55	64				
****** *	fold increase	1	53	73	100	240	1	13	15				

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Volume of distribution at steady state.

Observed maximum concentration.

⁵ Observed time to Cmax.

t_{1/2} term= half-life associated with the terminal phase of the concentration vs. time profile.

Area under the concentration versus time curve (extrapolated to infinity).

CL= serum clearance.

MRT= Mean residence time.

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For the pegylated anti-IL8 Fab' fragments, CL decreased by 46 to 180-fold. Terminal half-life and MRT-increased_14-to-35-fold_and_53-to-240-fold, respectively. For pegylated_anti-IL8_F(ab')₂_molecules, CL decreased 15 to 17-fold with pegylation, and terminal half-life and MRT increased by greater than 5-fold and 13-fold, respectively. The changes in these parameters increased for both pegylated Fab' and F(ab')₂ molecules with increasing PEG molecular weight and approached the values of the full-length anti-IL8 (terminal half-life of 74 hours, MRT of 99 hours and CL of 0.47 mL/hr/kg). In comparing the branched(1)40K Fab' (Group 6) and branched(1)40K F(ab')₂ (Group 4), unexpected pharmacokinetics were observed. The pegylated Fab' molecule appeared to remain in the serum longer than the pegylated F(ab')₂ (see Figure 66). The mean CL of branched(1)40K Fab' was 0.63 mL/hr/kg, but a higher CL was observed for branched(1)40kD F(ab')₂ (CL 0.92 mL/hr/kg). The terminal half-life, likewise, was longer for the Fab' than the F(ab')₂ pegylated molecule (110 vs 45 hours).

The pharmacokinetic data demonstrated that pegylation decreased CL and increased terminal t1/2 and MRT of anti-IL8 fragments (Fab' and F(ab')₂) to approach that of the full-length anti-IL8. Clearance was decreased with pegylation 46 to 180-fold for the Fab' and approximately 16-fold for the F(ab')₂. The terminal half-life of the Fab' anti-IL8 fragment was increased by 14 to 35-fold and approximately 5-fold for the F(ab')₂ anti-IL8. MRT, likewise, were extended by 53 to 240-fold for the Fab' and approximately 14-fold for the F(ab')₂. The branched(1) 40kD Fab' had a longer terminal half-life and lower clearance compared to the branched(1) 40kD F(ab')₂.

Y. IN VIVO EFFICACY TESTING OF ANTI-IL-8 ANTIBODY REAGENTS IN RABBIT MODEL OF ISCHEMIA/REPERFUSION AND ACID ASPIRATION-INDUCED ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

Full length murine anti-rabbit IL-8 monoclonal antibody 6G4.2.5, 40 kD branched PEG-6G4V11N35A Fab', and control antibody (anti-HIV gp120 monoclonal antibody 9E3.1F10) were tested in a rabbit ARDS model. The animals were weighed and anaesthetized by intramuscular injection of ketamine (50 mg/kg body weight), xylazine (5 mg/kg body weight), and acepromazine (0.75 mg/kg body weight). A second dose (20% of the first dosage) was given IM 15 minutes before removal of vascular clip, and third dose (60% of the first dosage) was given at tracheotomy. Intra-arterial catheter (22G, 1 in. Angiocath) and intra-venous catheter (24G, 1 in. angiocath) were be placed in the ear central artery and posterior marginal ear vein for blood samplings (arterial blood gases and CBC) and anti-IL-8 and fluid administration, respectively. The anaesthetized animals were transferred in a supine position to an operating tray; the abdominal area was shaved and prepared for surgery. Via a midline laparotomy, the superior mesenteric artery (SMA) was isolated and a microvascular arterial clip applied at the aortic origin. Before the temporary closure of the abdomen using 9 mm wound clip (Autoclip, Baxter), 15 ml of normal saline was

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given intraperitoneally as fluid supplement. After 110 minutes of intestinal ischemia, the abdominal incision was reopened and the arterial clip was released to allow reperfusion. Before closure, 5 ml of normal saline was given intraperitoneally for fluid replacement. The laparotomy incision was closed in two layers and the animals allowed to awaken.

After surgery, the animals were placed on a heating pad (38°C) and continuously monitored for up to 6 hours post reperfusion and lactated Ringer's 8-12 ml/kg/hr IV was given as fluid supplement.

At 22-24 hr post-reperfusion, a tracheotomy was performed under anesthesia. Normal physiologic saline was diluted 1:3 with water and adjusted to pH 1.5 (adjusted by using 1N HCL); 3 ml/kg body weight was then instilled intra-tracheally. Rectal temperature was maintained at 37 +/- 1 degree C using a homeothermic heat therapy pad (K-Mod II, Baxter). Fluid supplements (LRS) at a rate of 5 ml/kg/hour IV were given. Blood gases were monitored every hour. The rabbits were returned to the cage after 6 hr of continuous monitoring.

Just prior to aspiration, animals were treated with saline, the control monoclonal antibody (anti-HIV gp-120 IgG 9E3.1F10), the full length murine anti-rabbit IL8 (6g4.2.5 murine IgG2a anti-rabbit IL8) or the pegylated 6G4V11N35A Fab' (6G4V1N35A Fab' modified with 40kD branched PEG-maleimide as described in Section T above, denoted as "40 kD branched PEG-6G4V11N35A Fab' "). Data from saline or control antibody treated animals was combined and presented as "Control". Arterial blood gases and A-a PO2 gradient measurements were taken daily, and IV fluid supplementation was performed daily. A-a PO2 gradient was measured at 96 hr of reperfusion. The A-a PO2 gradient was calculated as:

A-a PO2 = [FIO2(PB - PH2O) - (PaCO2/RQ)] - PaO2.

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PaO2/FiO2 ratios were measured at 24hr and 48hr in room air and 100% oxygen.

After the final A-a PO2 gradient measurement, the animals were anesthetized with Nembutal 100mg/kg i.v. and the animals were euthanized by transecting the abdominal aorta in order to reduce red blood cell contamination of bronchoalveolar lavage fluid (BAL). The lungs were removed en bloc. The entire lung was weighed and then lavaged with an intratracheal tube (Hi-Lo tracheal tube, 3mm) using 30 ml of HBSS and lidocain. Total and differential leukocyte counts in the BAL were determined. Lesions/changes were verified by histological examination of each lobe of the right lung of each animal.

The gross lung weight, total leukocyte and polymorphonuclear cell counts in BAL, and PaO2/FiO2 data obtained are depicted in Figs. 67, 68 and 69, respectively. Treatment with 40 kD branched PEG-6G4V11N35A Fab' exhibited no effect on the biological parameters measured in the model as compared to the "Control" group. However, the data do not contradict the pharmacokinetic analysis or the in vitro activity analysis for the 40 kD branched PEG-6G4V11N35A Fab' presented in Sections (V) and (X) above. In addition, these data do not contradict the ability of the 40 kD branched PEG-6G4V11N35A Fab' to reach and act on disease effector targets in circulation or other tissues.

The following biological materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

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	<u>Material</u>	ATCC Accession No.	Deposit Date
	hybridoma cell line 5.12.14	HB 11553	February 15, 1993
	hybridoma cell line 6G4.2.5	HB 11722	September 28, 1994
5	pantiIL-8.2, E. coli strain 294 mm	97056	February 10, 1995
	p6G425chim2, E. coli strain 294 mm	97055	February 10, 1995
	p6G4V11N35A.F(ab') ₂	97890	February 20, 1997
	E. coli strain 49D6(p6G4V11N35A.F(ab') ₂	98332	February 20, 1997
	p6G425V11N35A.choSD	209552	December 16, 1997
10	clone#1933 aIL8.92 NB 28605/12	CRL-12444	December 11, 1997
	clone#1934 aIL8.42 NB 28605/14	CRL-12445	December 11, 1997

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These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable deposit for 30 years from the date of deposit. These cell lines will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the cell lines to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the cell lines to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if the deposited cell lines should be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a specimen of the same cell line. Availability of the deposited cell lines is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws

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	(ii)	TITLE OF INVENTION: Antibody Fragment-Polymer Conjugates and
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25		(F) ZIP: 94080
25	/a=\	COMPUTER READABLE FORM:
	(V)	(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
		(B) COMPUTER: IBM PC compatible
		(C) OPERATING SYSTEM: PC-DOS/MS-DOS
30		(D) SOFTWARE: WinPatin (Genentech)
	(vi)	CURRENT APPLICATION DATA:
		(A) APPLICATION NUMBER:
		(B) FILING DATE: 20-Feb-1998
35		(C) CLASSIFICATION:
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45	(2) IN	FORMATION FOR SEQ ID NO:1:
	(i)	SEQUENCE CHARACTERISTICS:
		(A) LENGTH: 22 base pairs
		(B) TYPE: Nucleic Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

(C) STRANDEDNESS: Single(D) TOPOLOGY: Linear

55

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CAGTCCAACT GTTCAGGACG CC 22

```
(2) INFORMATION FOR SEQ ID NO:2:
        (i) SEQUENCE CHARACTERISTICS:
5
            (A) LENGTH: 22 base pairs
            (B) TYPE: Nucleic Acid
            (C) STRANDEDNESS: Single
            (D) TOPOLOGY: Linear
10
      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
     GTGCTGCTCA TGCTGTAGGT GC 22
15
     (2) INFORMATION FOR SEQ ID NO:3:
        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 23 base pairs
20
            (B) TYPE: Nucleic Acid
            (C) STRANDEDNESS: Single
         (D) TOPOLOGY: Linear
       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
25
     GAAGTTGATG TCTTGTGAGT GGC 23
     (2) INFORMATION FOR SEQ ID NO:4:
30
        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: Nucleic Acid
            (C) STRANDEDNESS: Single
            (D) TOPOLOGY: Linear
35
       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
40
     GCATCCTAGA GTCACCGAGG AGCC 24
     (2) INFORMATION FOR SEQ ID NO:5:
        (i) SEQUENCE CHARACTERISTICS:
45
            (A) LENGTH: 22 base pairs
            (B) TYPE: Nucleic Acid
            (C) STRANDEDNESS: Single
            (D) TOPOLOGY: Linear
50
       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
     CACTGGCTCA GGGAAATAAC CC 22
   (2) INFORMATION FOR SEQ ID NO:6:
```

	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs
. 5	(B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
10	GGAGAGCTGG GAAGGTGTGC AC 22
	(2) INFORMATION FOR SEQ ID NO:7:
15	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear
20	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
	ACAAACGCGT ACGCTGACAT CGTCATGACC CAGTC 35
25	(2) INFORMATION FOR SEQ ID NO:8:
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 base pairs (B) TYPE: Nucleic Acid
30	(C) STRANDEDNESS: Single (D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
35	ACAAACGCGT ACGCTGATAT TGTCATGACT CAGTC 35
	(2) INFORMATION FOR SEQ ID NO:9:
40	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single
45	(D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
50	ACAAACGCGT ACGCTGACAT CGTCATGACA CAGTC 35
	(2) INFORMATION FOR SEQ ID NO:10:
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 37 base pairs
55	(B) TYPE: Nucleic Acid

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(D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10: 5 GCTCTTCGAA TGGTGGGAAG ATGGATACAG TTGGTGC 37 (2) INFORMATION FOR SEQ ID NO:11: 10 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 39 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11: CGATGGGCCC GGATAGACCG ATGGGGCTGT TGTTTTGGC 39 20 (2) INFORMATION FOR SEQ ID NO:12: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 39 base pairs (B) TYPE: Nucleic Acid 25 (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12: 30 CGATGGGCCC GGATAGACTG ATGGGGCTGT CGTTTTGGC 39 (2) INFORMATION FOR SEQ ID NO:13: 35 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 39 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single 40 (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13: 45 CGATGGGCCC GGATAGACGG ATGGGGCTGT TGTTTTGGC 39 (2) INFORMATION FOR SEQ ID NO:14: (i) SEQUENCE CHARACTERISTICS: 50 (A) LENGTH: 39 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

55

CGATGGGCCC GGATAGACAG ATGGGGCTGT TGTTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:15: 5 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 39 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15: CGATGGGCCC GGATAGACCG ATGGGGCTGT TGTTTTGGC 39 15 (2) INFORMATION FOR SEQ ID NO:16: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 39 base pairs 20 (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16: 25 CGATGGGCCC GGATAGACTG ATGGGGCTGT TGTTTTGGC 39 (2) INFORMATION FOR SEQ ID NO:17: 30 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 39 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single 35 (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17: 40 CGATGGGCCC GGATAGACAG ATGGGGCTGT TGTTTTGGC 39 (2) INFORMATION FOR SEQ ID NO:18: (i) SEQUENCE CHARACTERISTICS: 45 (A) LENGTH: 39 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18: CGATGGGCCC GGATAGACGG ATGGGGCTGT TGTTTTGGC 39 55 (2) INFORMATION FOR SEQ ID NO:19:

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	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 369 base pairs (B) TYPE: Nucleic Acid	
5	(C) STRANDEDNESS: Double	
	(D) TOPOLOGY: Linear	
	(with apparence perapapaton, see TD NO.19.	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:	
10		
	GACATTGTCA TGACACAGTC TCAAAAATTC ATGTCCACAT CAGTAGGAGA 50	
	CAGGGTCAGC GTCACCTGCA AGGCCAGTCA GAATGTGGGT ACTAATGTAG 100	
	CAGGGTCAGC GICACCIGCA AGGCCAGICA GAAIGIGGGI ACIAAIGIAG 100	
15	CCTGGTATCA ACAGAAACCA GGGCAATCTC CTAAAGCACT GATTTACTCG 150	
	TCATCCTACC GGTACAGTGG AGTCCCTGAT CGCTTCACAG GCAGTGGATC 200	
	TGGGACAGAT TTCACTCTCA CCATCAGCCA TGTGCAGTCT GAAGACTTGG 250	
20		
,	CAGACTATTT CTGTCAGCAA TATAACATCT ATCCTCTCAC GTTCGGTCCT 300	
	GGGACCAAGC TGGAGTTGAA ACGGGCTGAT GCTGCACCAC CAACTGTATC 350	
	GGGACCARGE Idealorian ideadorian delectronic delectron	
25	CATCTTCCCA CCATTCGAA 369	
	(2) INFORMATION FOR SEQ ID NO:20:	
	(2) INFORMATION FOR SEQ ID NO:20:	
	(i) SEQUENCE CHARACTERISTICS:	
30		
	(B) TYPE: Amino Acid (D) TOPOLOGY: Linear	
	(b) Toronogi. Dinear	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:	
35		
	Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val 1 5 10 15	
	Gly Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly	
40	20 25 30	
	Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys	
	35 40 45	
45		
	50 55 60	
	Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile	
	65 70 75	
50		
	Ser His Val Gln Ser Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln 80 85 90	
	Tyr Asn Ile Tyr Pro Leu Thr Phe Gly Pro Gly Thr Lys Leu Glu	
55	95 100 105	

	Leu Lys Arg Ala Asp Ala Ala Pro Pro Thr Val Ser Ile Phe Pro 110 115 120
5	Pro Phe Glu 123
	(2) INFORMATION FOR SEQ ID NO:21:
10	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 417 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
	TTCTATTGCT ACAAACGCGT ACGCTGAGGT GCAGCTGGTG GAGTCTGGGG 50
20	GAGGCTTAGT GCCGCCTGGA GGGTCCCTGA AACTCTCCTG TGCAGCCTCT 100
	GGATTCATAT TCAGTAGTTA TGGCATGTCT TGGGTTCGCC AGACTCCAGG 150
	CAAGAGCCTG GAGTTGGTCG CAACCATTAA TAATAATGGT GATAGCACCT 200
25	ATTATCCAGA CAGTGTGAAG GGCCGATTCA CCATCTCCCG AGACAATGCC 250
	AAGAACACCC TGTACCTGCA AATGAGCAGT CTGAAGTCTG AGGACACAGC 300
30	CATGTTTTAC TGTGCAAGAG CCCTCATTAG TTCGGCTACT TGGTTTGGTT
•	ACTGGGGCCA AGGGACTCTG GTCACTGTCT CTGCAGCCAA AACAACAGCC 400
35	CCATCTGTCT ATCCGGG 417 (2) INFORMATION FOR SEQ ID NO:22:
40	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 130 amino acids (B) TYPE: Amino Acid (D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
45	Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Pro Pro Gly
	1 5 10 15 Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Ser 20 25 30
50	Ser Tyr Gly Met Ser Trp Val Arg Gln Thr Pro Gly Lys Ser Leu 35 40 45
55	Glu Leu Val Ala Thr Ile Asn Asn Asn Gly Asp Ser Thr Tyr Tyr 50 55 60

	Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala 65 70 75
•	
5	Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp 80 85 90
	Thr Ala Met Phe Tyr Cys Ala Arg Ala Leu Ile Ser Ser Ala Thr 95 100 105
10	Trp Phe Gly Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala 110 115 120
15	Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro 125 130
	(2) INFORMATION FOR SEQ ID NO:23:
20	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 31 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single
	(D) TOPOLOGY: Linear
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
	ACAAACGCGT ACGCTGATAT CGTCATGACA G 31
30	(2) INFORMATION FOR SEQ ID NO:24:
	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 31 base pairs(B) TYPE: Nucleic Acid(C) STRANDEDNESS: Single
35	(D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
40	GCAGCATCAG CTCTTCGAAG CTCCAGCTTG G 31
	(2) INFORMATION FOR SEQ ID NO:25:
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: Nucleic Acid
	(C) STRANDEDNESS: Single (D) TOPOLOGY: Linear
50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
	CCACTAGTAC GCAAGTTCAC G 21

(2) INFORMATION FOR SEQ ID NO:26:

55

- WO 98/37200 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 33 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26: GATGGGCCCT TGGTGGAGGC TGCAGAGACA GTG 33 10 (2) INFORMATION FOR SEQ ID NO:27: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 714 base pairs 15 (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27: 20 25
- ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT 50 TGCTACAAAC GCGTACGCTG ATATCGTCAT GACACAGTCT CAAAAATTCA 100 TGTCCACATC AGTAGGAGAC AGGGTCAGCG TCACCTGCAA GGCCAGTCAG 150 AATGTGGGTA CTAATGTAGC CTGGTATCAA CAGAAACCAG GGCAATCTCC 200 30 TARAGCACTG ATTTACTCGT CATCCTACCG GTACAGTGGA GTCCCTGATC 250 GCTTCACAGG CAGTGGATCT GGGACAGATT TCACTCTCAC CATCAGCCAT 300 GTGCAGTCTG AAGACTTGGC AGACTATTTC TGTCAGCAAT ATAACATCTA 350 35 TCCTCTCACG TTCGGTCCTG GGACCAAGCT GGAGCTTCGA AGAGCTGTGG 400 CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA GTTGAAATCT 450 40 GGAACTGCTT CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC 500 CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG 550 AGAGTGTCAC AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC 600 45 ACCCTGACGC TGAGCAAAGC AGACTACGAG AAACACAAAG TCTACGCCTG 650 CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA 700 50 GGGGAGAGTG TTAA 714
 - (2) INFORMATION FOR SEQ ID NO:28:
- 55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 237 amino acids

DESCRIPTION AND DESCRIPTION AND I S

(B) TYPE: Amino Acid(D) TOPOLOGY: Linear

•			·																
		(x:	L) SI	EQUE	VCE I	DESCI	(IPT	ON:	SEQ	ID	10:28	3:				····		 1.1111	
	5	Met 1	Lys	Lys	Asn	Ile 5	Ala	Phe	Leu	Leu	Ala 10	Ser	Met	Phe	Val	Phe 15			
	10	Ser	Ile	Ala	Thr	Asn 20	Ala	Tyr	Ala	Asp	Ile 25	Val	Met	Thr	Gln	Ser 30			
		Gln	Lys	Phe	Met	Ser 35	Thr	Ser	Val	Gly	Asp 40	Arg	Val	Ser	Val	Thr 45			
	15	Cys	Lys	Ala	Ser	Gln 50	Asn	Val	Gly	Thr	Asn 55	Val	Ala	Trp	Tyr	Gln 60			
		Gln	Lys	Pro	Gly	Gln 65	Ser	Pro	Lys	Ala	Leu 70	Ile	Tyr	Ser	Ser	Ser 75			
	20	Tyr	Arg	Tyr	Ser	Gly 80	Val	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser 90			
	25	Gly	Thr	Asp	Phe	Thr 95	Leu	Thr	Ile	Ser	His 100	Val	Gln	Ser	Glu	Asp 105			
		Leu	Ala	Asp	Tyr	Phe 110	Cys	Gln	Gln	Tyr	Asn 115	Ile	Tyr	Pro	Leu	Thr 120			
•	30	Phe	Gly	Pro	Gly	Thr 125	Lys	Leu	Glu	Leu	Arg 130	Arg	Ala	Val	Ala	Ala 135			
	25	Pro	Ser	Val	Phe	Ile 140	Phe	Pro	Pro	Ser	Asp 145	Glu	Gln	Leu	Lys	Ser 150			
	35	Gly	Thr	Ala	Ser	Val 155	Val	Cys	Leu	Leu	Asn 160		Phe	Tyr	Pro	Arg 165			
	40	Glu	Ala	Lys	Val	Gln 170		Lys	Val	Asp	Asn 175		Leu	Gln	Ser	Gly 180			
		Asn	Ser	Gln	Glu	Ser 185		Thr	Glu	Gln	Asp 190		Lys	Asp	Ser	Thr 195			
	45	Tyr	Ser	Leu	Ser	Ser 200		Leu	Thr	Leu	Ser _ 205		Ala	Asp	туг	Glu 210			
		Lys	His	Lys	Val	Tyr 215	•	. Cys	Glu	Val	Thr 220		Glr.	Gly	Leu	Ser 225	•	-	
	50	Ser	Pro	Val	Thr	Lys 230		Phe	: Asn	Arg	Gly 235		237						
	55					FOR													
		(i) S	EQUE	NCE	CHAR	ACTE	RIST	CICS:										

	(B) TYPE: Nucleic Acid (C) STRANDEDNESS: Double												
	(D) TOPOLOGY: Linear												
5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:												
10	ATGAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT 50												
10	TGCTACAAAC GCGTACGCTG AGGTGCAGCT GGTGGAGTCT GGGGGAGGCT 100												
	TAGTGCCGCC TGGAGGGTCC CTGAAACTCT CCTGTGCAGC CTCTGGATTC 150												
15	ATATTCAGTA GTTATGGCAT GTCTTGGGTT CGCCAGACTC CAGGCAAGAG 200												
	CCTGGAGTTG GTCGCAACCA TTAATAATAA TGGTGATAGC ACCTATTATC 250												
20	CAGACAGTGT GAAGGGCCGA TTCACCATCT CCCGAGACAA TGCCAAGAAC 300												
	ACCCTGTACC TGCAAATGAG CAGTCTGAAG TCTGAGGACA CAGCCATGTT 350												
	TTACTGTGCA AGAGCCCTCA TTAGTTCGGC TACTTGGTTT GGTTACTGGG 400												
25	GCCAAGGGAC TCTGGTCACT GTCTCTGCAG CCTCCACCAA GGGCCCATCG 450												
	GTCTTCCCCC TGGCACCCTC CTCCAAGAGC ACCTCTGGGG GCACAGCGGC 500												
30	CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCGGTG ACGGTGTCGT 550												
	GGAACTCAGG CGCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCCTA 600												
	CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG 650												
35	CAGCTTGGGC ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGCA 700												
	ACACCAAGGT GGACAAGAAA GTTGAGCCCA AATCTTGTGA CAAAACTCAC 750												
40	ACATGA 756												
	(2) INFORMATION FOR SEQ ID NO:30:												
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 251 amino acids												
45	(B) TYPE: Amino Acid (D) TOPOLOGY: Linear												
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:												
50	Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe 1 5 10 15												
	Ser Ile Ala Thr Asn Ala Tyr Ala Glu Val Gln Leu Val Glu Ser 20 25 30												
55	Gly Gly Gly Leu Val Pro Pro Gly Gly Ser Leu Lys Leu Ser Cys												

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(A) LENGTH: 756 base pairs

35 40 45

•		Ala	Ala	Ser	Gly		Ile	Phe	Ser	Ser		Gly	Met	Ser	Trp	
	•					50					55					60
	5	Arg	Gln	Thr	Pro		Lys	Ser	Leu	Glu		Val	Ala	Thr	Ile	
						65					70					75
		Asn	Asn	Gly	Asp		Thr	Tyr	Tyr	Pro		Ser	Val	Lys	Gly	Arg 90
	10					80					85					90
		Phe	Thr	Ile	Ser		Asp	Asn	Ala	Lys	Asn 100	Thr	Leu	Tyr	Leu	Gln 105
						95										
	15	Met	Ser	Ser	Leu	Lys 110	Ser	Glu	Asp	Thr	Ala 115	Met	Phe	Tyr	Cys	Ala 120
		Arg	Ala	Leu	Ile	Ser 125	Ser	Ala	Thr	Trp	Phe 130	Gly	Tyr	Trp	Gly	Gln 135
	20												_		_	
*********		Gly	Thr	Leu	Val	Thr 140	Val	Ser	Ala	Ala	Ser 145	Thr	Lys	GTA	Pro	ser 150
					_		_	_	_	_				~ 3	~3	mb
	25	Val	Phe	Pro	Leu	155	Pro	Ser	Ser	гуs	160	Thr	Ser	GIY	GIY	165
			77-	T 011	Gly	Crea	T 011	1701	T 2.00	7 cm	The sac	Dhe	Pro	Glu	Pro	Val
		AIA	AIA	Dea	Gry	170	Deu	VAI	пуз	rap	175	7110	110	014		180
	30	Thr	Val	Ser	Trp	Asn	Ser	Glv	Ala	Leu	Thr	Ser	Gly	Val	His	Thr
	30	1111	,,,,			185		2			190		-			195
		Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser
	25					200					205					210
	35	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile
						215					220					225
		Cys	Asn	Val	Asn		Lys	Pro	Ser	Asn			. Val	Asp	Lys	Lys
	40					230					235					240
		Val	Glu	Pro	Lys		Cys	Asp	Lys	Thr						
						245					250	251	•			
	45	(2)	INFO	RMAT	ION :	FOR :	SEQ	ID N	0:31	:						

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

55 CAGTCCAACT GTTCAGGACG CC 22

50

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	(2) INFORMATION FOR SEQ ID NO:32:	
5	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear 	
10	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:	
	GTGCTGCTCA TGCTGTAGGT GC 22	
15	(2) INFORMATION FOR SEQ ID NO:33:	
20	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear 	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:	· · · · · · · · · · · · · · · · · · ·
25	GAAGTTGATG TCTTGTGAGT GGC 23	
	(2) INFORMATION FOR SEQ ID NO:34:	
30	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 24 base pairs(B) TYPE: Nucleic Acid(C) STRANDEDNESS: Single(D) TOPOLOGY: Linear	
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:	
40	GCATCCTAGA GTCACCGAGG AGCC 24 (2) INFORMATION FOR SEQ ID NO:35:	
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single	
. 50	(D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:	
	CACTGGCTCA GGGAAATAAC CC 22	
55	(2) INFORMATION FOR SEQ ID NO:36:	
	(i) SEQUENCE CHARACTERISTICS:	

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	(A) LENGTH: 22 base pairs(B) TYPE: Nucleic Acid(C) STRANDEDNESS: Single	
5	(D) TOPOLOGY: Linear	
J	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:	
10	GGAGAGCTGG GAAGGTGTGC AC 22	
	(2) INFORMATION FOR SEQ ID NO:37:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 37 base pairs	
15	(B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single	
	(D) TOPOLOGY: Linear	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:	
20		
	CCAATGCATA CGCTGACATC GTGATGACCC AGACCCC 37	
	(2) INFORMATION FOR SEQ ID NO:38:	
25	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 37 base pairs	
	(B) TYPE: Nucleic Acid	
	(C) STRANDEDNESS: Single	
30	(D) TOPOLOGY: Linear	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:	
35	CCAATGCATA CGCTGATATT GTGATGACTC AGACTCC 37	
	(2) INFORMATION FOR SEQ ID NO:39:	
	(i) SEQUENCE CHARACTERISTICS:	
40	(A) LENGTH: 37 base pairs	
	(B) TYPE: Nucleic Acid	·
	(C) STRANDEDNESS: Single (D) TOPOLOGY: Linear	
45	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:	
	CCAATGCATA CGCTGACATC GTGATGACAC AGACACC 37	,
50	(2) INFORMATION FOR SEQ ID NO:40:	
	(i) SEQUENCE CHARACTERISTICS:	•
	(A) LENGTH: 35 base pairs	
<i>e e</i>	(B) TYPE: Nucleic Acid	
55	(C) STRANDEDNESS: Single (D) TOPOLOGY: Linear	

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-	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:	
5	AGATGTCAAT TGCTCACTGG ATGGTGGGAA GATGG 35	
	(2) INFORMATION FOR SEQ ID NO:41:	
	(i) SEQUENCE CHARACTERISTICS:	
10	(A) LENGTH: 32 base pairs	
	(B) TYPE: Nucleic Acid	
	(C) STRANDEDNESS: Single	
	(D) TOPOLOGY: Linear	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:	
	CAAACGCGTA CGCTGAGATC CAGCTGCAGC AG 32	
20	(2) INFORMATION FOR SEQ ID NO:42:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 32 base pairs	. mr. 44
	(B) TYPE: Nucleic Acid	
25	(C) STRANDEDNESS: Single	
	(D) TOPOLOGY: Linear	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:	
30	CAAACGCGTA CGCTGAGATT CAGCTCCAGC AG 32	
	(2) INFORMATION FOR SEQ ID NO:43:	•
35	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 39 base pairs	
	(B) TYPE: Nucleic Acid	
	(C) STRANDEDNESS: Single	
40	(D) TOPOLOGY: Linear	
40	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:	
45	CGATGGGCCC GGATAGACCG ATGGGGCTGT TGTTTTGGC 39	
	(2) INFORMATION FOR SEQ ID NO:44:	
	//\	
	(i) SEQUENCE CHARACTERISTICS:	
50	(A) LENGTH: 39 base pairs (B) TYPE: Nucleic Acid	
30	(C) STRANDEDNESS: Single	
	(D) TOPOLOGY: Linear	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:	
55		
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CGATGGGCCC GGATAGACTG ATGGGGCTGT TGTTTTGGC 39

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(2) INFORMATION FOR SEQ ID NO:45:
  5
         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 39 base pairs
             (B) TYPE: Nucleic Acid
             (C) STRANDEDNESS: Single
             (D) TOPOLOGY: Linear
 10
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
       CGATGGGCCC GGATAGACAG ATGGGGCTGT TGTTTTGGC 39
15
      (2) INFORMATION FOR SEQ ID NO:46:
         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 39 base pairs
             (B) TYPE: Nucleic Acid
 20
             (C) STRANDEDNESS: Double
             (D) TOPOLOGY: Linear
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:
 25
       CGATGGGCCC GGATAGACGG ATGGGGCTGT TGTTTTGGC 39
      (2) INFORMATION FOR SEQ ID NO:47:
 30
         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 391 base pairs
             (B) TYPE: Nucleic Acid
             (C) STRANDEDNESS: Double
 35
             (D) TOPOLOGY: Linear
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:
       GATATCGTGA TGACACAGAC ACCACTCTCC CTGCCTGTCA GTCTTGGAGA 50
 40
       TCAGGCCTCC ATCTCTTGCA GATCTAGTCA GAGCCTTGTA CACGGTATTG 100
       GAAACACCTA TTTACATTGG TACCTGCAGA AGCCAGGCCA GTCTCCAAAG 150
 45
       CTCCTGATCT ACAAAGTTTC CAACCGATTT TCTGGGGTCC CAGACAGGTT 200
       CAGTGGCAGT GGATCAGGGA CAGATTTCAC ACTCAGGATC AGCAGAGTGG 250
 50
       AGGCTGAGGA TCTGGGACTT TATTTCTGCT CTCAAAGTAC ACATGTTCCG 300
       CTCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAACGGG CTGATGCTGC 350
       ACCAACTGTA TCCATCTTCC CACCATCCAG TGAGCAATTG A 391
 55
      (2) INFORMATION FOR SEQ ID NO:48:
```

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•	(i) SEQUENCE CHARACTERISTICS:(A)_LENGTH:_131_amino_acids
5	(B) TYPE: Amino Acid (D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:
10	Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu 1 5 10 15
	Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val 20 25 30
15	His Gly Ile Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro 35 40 45
20	Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe 50 55 60
	Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp 65 70 75
25	Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Leu 80 85 90
	Tyr Phe Cys Ser Gln Ser Thr His Val Pro Leu Thr Phe Gly Ala 95 100 105
30	Gly Thr Lys Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro Thr Val 110 115 120
35	Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Lys 125 130 131
	(2) INFORMATION FOR SEQ ID NO:49: (i) SEQUENCE CHARACTERISTICS:
- 40	(A) LENGTH: 405 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Double (D) TOPOLOGY: Linear
45	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:
	GAGATTCAGC TGCAGCAGTC TGGACCTGAG CTGATGAAGC CTGGGGCTTC 50
50	AGTGAAGATA TCCTGCAAGG CTTCTGGTTA TTCATTCAGT AGCCACTACA 100
	TGCACTGGGT GAAGCAGAGC CATGGAAAGA GCCTTGAGTG GATTGGCTAC 150
	ATTGATCCTT CCAATGGTGA AACTACTTAC AACCAGAAAT TCAAGGGCAA 200
55	GGCCACATTG ACTGTAGACA CATCTTCCAG CACAGCCAAC GTGCATCTCA 250

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GCAGCCTGAC ATCTGATGAC TCTGCAGTCT ATTTCTGTGC AAGAGGGGAC 300 TATAGATACA ACGGCGACTG GTTTTTCGAT GTCTGGGGNG NAGGGACCAC 350 GGTCACCGTC TCCTCCGCCA AAACCGACAG CCCCATCGGT CTATCCGGGC 400 5 CCATC 405 (2) INFORMATION FOR SEQ ID NO:50: 10 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 135 amino acids (B) TYPE: Amino Acid (D) TOPOLOGY: Linear 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50: Glu Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Met Lys Pro Gly 20 Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Ser Ser His Tyr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu 25 Glu Trp Ile Gly Tyr Ile Asp Pro Ser Asn Gly Glu Thr Thr Tyr 55 Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser 30 Ser Ser Thr Ala Asn Val His Leu Ser Ser Leu Thr Ser Asp Asp 35 Ser Ala Val Tyr Phe Cys Ala Arg Gly Asp Tyr Arg Tyr Asn Gly 95 100 Asp Trp Phe Phe Asp Val Trp Gly Xaa Gly Thr Thr Val Thr Val 40 110 Ser Ser Ala Lys Thr Asp Ser Pro Ile Gly Leu Ser Gly Pro Ile 130 125 (2) INFORMATION FOR SEQ ID NO:51: 45 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs (B) TYPE: Nucleic Acid 50 (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

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CTTGGTGGAG GCGGAGGAGA CG 22

•	(2) INFORMATION FOR SEQ ID NO:52:
5	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 38 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear
10	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:
	GAAACGGGCT GTTGCTGCAC CAACTGTATT CATCTTCC 38
15	(2) INFORMATION FOR SEQ ID NO:53:
20	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 31 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single
	(D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:
25	GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 31
	(2) INFORMATION FOR SEQ ID NO:54:
30	 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:
40	CTTGGTGGAG GCGGAGGAGA CG 22 (2) INFORMATION FOR SEQ ID NO:55:
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 729 base pairs (B) TYPE: Nucleic Acid
	(C) STRANDEDNESS: Double (D) TOPOLOGY: Linear
50	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:
	ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT 50
55	TGCTACAAAT GCATACGCTG ATATCGTGAT GACACAGACA CCACTCTCCC 100
دد	TGCCTGTCAG TCTTGGAGAT CAGGCCTCCA TCTCTTGCAG ATCTAGTCAG 150

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•	AGCCTTGTAC ACGGTATTGG AAACACCTAT TTACATTGGT ACCTGCAGAA 200
	GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC AACCGATTTT 250
5	CTGGGGTCCC AGACAGGTTC AGTGGCAGTG GATCAGGGAC AGATTTCACA 300
	CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC 350
10	TCAAAGTACA CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC 400
	TGAAACGGGC TGTTGCTGCA CCAACTGTAT TCATCTTCCC ACCATCCAGT 450
15	GAGCAATTGA AATCTGGAAC TGCCTCTGTT GTGTGCCTGC TGAATAACTT 500
.5	CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC GCCCTCCAAT 550
	CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC 600
20	TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA 650
*****	CAAAGTCTAC GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA 700
25	CAAAGAGCTT CAACAGGGGA GAGTGTTAA 729
	(2) INFORMATION FOR SEQ ID NO:56:
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 242 amino acids
30	(B) TYPE: Amino Acid (D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:
35	Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe
	1 5 10 15
	Ser Ile Ala Thr Asn Ala Tyr Ala Asp Ile Val Met Thr Gln Thr 20 25 30
40	Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
	35 40 45
45	Cys Arg Ser Ser Gln Ser Leu Val His Gly Ile Gly Asn Thr Tyr 50 55 60
	Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu
	65 70 75
50	Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe 80 85 90
50	80 85 90 Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile Ser Arg
50	80 85 90

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	110 115 120
•	His Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
£	125 130 135
5	Arg Ala Val Ala Ala Pro Thr Val Phe Ile Phe Pro Pro Ser Ser
	240
10	Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn 155 160 165
10	Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
	170 175 180
15	Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
	185 190 195
	Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser 200 205 210
20	
	Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr 215 220 225
	His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly
25	230 235 240
	Glu Cys
	242
30	(2) INFORMATION FOR SEQ ID NO:57:
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 762 base pairs
	(B) TYPE: Nucleic Acid
35	(C) STRANDEDNESS: Double (D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:
40	
40	ATGAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT 50
	TGCTACAAAC GCGTACGCTG AGATTCAGCT GCAGCAGTCT GGACCTGAGC 100
45	TGATGAAGCC TGGGGCTTCA GTGAAGATAT CCTGCAAGGC TTCTGGTTAT 150
	TCATTCAGTA GCCACTACAT GCACTGGGTG AAGCAGAGCC ATGGAAAGAG 200
	CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA ACTACTTACA 250
50	
	ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC 300
	ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA 350
55	TTTCTGTGCA AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG 400

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	TCTGGGGCGC AGGGACCACG GTCACCGTCT CCTCCGCCTC CACCAAGGGC 450	
V W ST WY ANDRE MANAGEMENT	CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC 500	
5	AGCGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG 550	
	TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT 600	
10	GTCCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC 650	
10	CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC 700	
	CCAGCAACAC CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA 750	
15	ACTCACACAT GA 762	
	(2) INFORMATION FOR SEQ ID NO:58:	
20	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 253 amino acids	
	(B) TYPE: Amino Acid (D) TOPOLOGY: Linear	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:	
	Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe 1 5 10 15	
20	Ser Ile Ala Thr Asn Ala Tyr Ala Glu Ile Gln Leu Gln Gln Ser	
30	20 25 30	
	Gly Pro Glu Leu Met Lys Pro Gly Ala Ser Val Lys Ile Ser Cys 35 40 45	
35	Lys Ala Ser Gly Tyr Ser Phe Ser Ser His Tyr Met His Trp Val 50 55 60	
	Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly Tyr Ile Asp	
40	65 70 75	
	Pro Ser Asn Gly Glu Thr Thr Tyr Asn Gln Lys Phe Lys Gly Lys 80 85 90	
	Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Asn Val His	
45	95 100 105	
	Leu Ser Ser Leu Thr Ser Asp Asp Ser Ala Val Tyr Phe Cys Ala 110 115 120	
50	Arg Gly Asp Tyr Arg Tyr Asn Gly Asp Trp Phe Phe Asp Val Trp	
	125 130 135	
	Gly Ala Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly 140 145 150	
55	Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly	

160 165 155 Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu 170 5 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val 185 His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu 200 10 Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr 215 Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp 15 235 230 Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr 250 20 (2) INFORMATION FOR SEQ ID NO:59: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 114 amino acids (B) TYPE: Amino Acid 25 (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59: Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu 30 10 Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val 35 His Gly Ile Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Tyr Lys Val Ser Asn Arg 40 Phe Ser Gly Val Pro Asp Arg Phe Ser Asp Ser Gly Ser Gly Thr 70 Asp Phe Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Leu Gly 45 85 Leu Tyr Phe Cys Ser Gln Ser Thr His Val Pro Leu Thr Phe Gly 100 50 Ala Gly Thr Lys Leu Glu Leu Lys Arg (2) INFORMATION FOR SEQ ID NO:60: 55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114 amino acids

(B) TYPE: Amino Acid (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60: 5 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Ser Leu Val 10 20 His Gly Ile Gly Asn Thr Tyr Leu His Trp Tyr Gln Gln Lys Pro 15 Gly Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr 20 Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Ser Gln Ser Thr His Val Pro Leu Thr Phe Gly 25 100 95 Gln Gly Thr Lys Val Glu Ile Lys Arg 110 30 (2) INFORMATION FOR SEQ ID NO:61: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 109 amino acids (B) TYPE: Amino Acid 35 (D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61: Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val 40 1 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Thr Ile Ser 45 Lys Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Ser Gly Ser Thr Leu Glu Ser Gly Val Pro 50 50 Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr 70 Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln 55

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	Gln His Asn Glu Tyr Pro Leu Thr Phe Gly Gln Gly Thr Lys Val 95 100 105	
5		
	(2) INFORMATION FOR SEQ ID NO:62:	
10	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 117 amino acids(B) TYPE: Amino Acid(D) TOPOLOGY: Linear	
15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:	
	Glu Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Met Lys Pro Gly 1 5 10 15	
20	Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Ser 20 25 30	
	Ser His Tyr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu 35 40 45	
25	Glu Trp Ile Gly Tyr Ile Asp Pro Ser Asn Gly Glu Thr Thr Tyr 50 55 60	
30	Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser 65 70 75	·
	Ser Ser Thr Ala Asn Val His Leu Ser Ser Leu Thr Ser Asp Asp 80 85 90	•
35	95 100 105	
40	Gly Asp Trp Phe Phe Asp Val Trp Gly Ala Gly Thr 110 115 117	
40	(2) INFORMATION FOR SEQ ID NO:63:	
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 117 amino acids (B) TYPE: Amino Acid (D) TOPOLOGY: Linear	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:	
50	and the state of t	
	Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Ser Phe Ser	
55	Ser His Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu	·

								•
			35		40		1	45
	Glu Trp	Val Gly		Asp Pro	Ser Asn	Gly Glu	Thr Thr	
5	Asn Gln	Lys Phe	50 Lys Gly 65	Arg Phe	55 Thr Ile 70	Ser Arg	Asp Asn	60 Ser 75
10	Lys Asn	Thr Leu	Tyr Leu 80	Gln Met	Asn Ser 85	Leu Arg	Ala Glu	Asp 90
	Thr Ala	Val Tyr	Tyr Cys 95	Ala Ala	Arg Gly 100	Asp Tyr	Arg Tyr	Asn 105
15	Gly Asp	Trp Phe	Phe Asp	Val Trp	Gly Gln 115	Gly Thr 117		
	(2) INFO	RMATION	FOR SEQ	ID NO:64	1 :			
20	·	-	CHARACTE H: 116 a					
	•	-	Amino A OGY: Lin					
25	(xi) SI	EQUENCE	DESCRIPT	ION: SEC	Q ID NO:6	4:		
	Glu Val	Gln Leu	Val Glu 5	Ser Gly	y Gly Gly 10	Leu Val	Gln Pro	Gly 15
30	Gly Ser	Leu Arg	Leu Ser 20	Cys Ala	a Ala Ser 25	Gly Phe	Ser Phe	Thr 30
35	Gly His	Trp Met	Asn Trp 35	Val Arg	g Gln Ala 40		Lys Gly	Leu 45
	Glu Trp	Val Gly	Met Ile 50	His Pro	o Ser Asp 55		Thr Arg	Tyr 60
40	Ala Asp	Ser Val	Lys Gly 65	Arg Ph	e Thr Ile 70		Asp Asn	Ser 75
	Lys Asn	Thr Leu	Tyr Leu 80	Gln Me	t Asn Ser 85		Ala Glu	Asp 90
45	Thr Ala	Val Tyr	Tyr Cys	Ala Al	a Arg Gly		Phe Tyr	Gly 105
	Thr Thr	Tyr Phe	Asp Tyr	Trp Gl	y Gln Gly	Thr		·

110 115 116

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- (2) INFORMATION FOR SEQ ID NO:65:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 242 amino acids
 - (B) TYPE: Amino Acid
 - (D) TOPOLOGY: Linear

(xi) SEQUENCE	DESCRIPTION:	SEQ	ID	NO:65:
---------------	--------------	-----	----	--------

•		,		_	•										·					
	5	Met 1	Lys	Lys	Asn	Ile 5	Ala	Phe	Leu	Leu	Ala 10	Ser	Met	Phe	Val	Phe 15	ALL PARTY OF THE P	AV-	Omnibility Schooling Lagrange 2 July	As contacts de accessed
		Ser	Ile	Ala	Thr	Asn 20	Ala	Tyr	Ala	Asp	Ile 25	Gln	Met	Thr	Gln	Ser 30				
	10	Pro	Ser	Ser	Leu	Ser 35	Ala	Ser	Val	Gly	Asp 40	Arg	Val	Thr	Ile	Thr 45				
		Cys	Arg	Ser	Ser	Gln 50	Ser	Leu	Val	His	Gly 55	Ile	Gly	Asn	Thr	Tyr 60				
	15	Leu	His	Trp	Tyr	Gln 65	Gln	Lys	Pro	Gly	Lys 70	Ala	Pro	Lys	Leu	Leu 75				
	20	Ile	туr	Lys	Val	Ser 80	Asn	Arg	Phe	Ser	Gly 85	Val	Pro	Ser	Arg	Phe 90				
		Ser	Gly	Ser	Gly	Ser 95	Gly	Thr	Asp	Phe	Thr 100	Leu	Thr	Ile	Ser	Ser 105				
	25	Leu	Gln	Pro	Glu	Asp 110	Phe	Ala	Thr	Tyr	Tyr 115	Cys	Ser	Gln	Ser	Thr 120				
		His	Val	Pro	Leu	Thr 125	Phe	Gly	Gln	Gly	Thr 130		Val	Glu	Ile	Lys 135				
	30	Arg	Thr	Val	Ala	Ala 140	Pro	Ser	Val	Phe	Ile 145	Phe	Pro	Pro	Ser	Asp 150				
	35	Glu	Gln	Leu	Lys	Ser 155	Gly	Thr	Ala	Ser	Val 160	Val	. Cys	Lev	. Lev	Asn 165				
		Asn	Phe	туг	Pro	Arg 170		Ala	Lys	Val	Gln 175		Lys	val	l Asp	180				
	40	Ala	Leu	Gln	Ser	Gly 185		Ser	Gln	Glu	Ser 190		LThi	Glv	ı Glr	195				
	45	Ser	Lys	Asp	Ser	Thr 200		Ser	Leu	Ser	Ser 205		r Let	ı Thi	r Le	210		:		
	45	Lvs	Ala	Asp	Tyr	Glu	Lys	His	Lys	val	Tyı	Ala	а Су	s Gl	u Va	Thr		ŭ.		
						215					220)				225 g Gly				
	50				Leu	230			, ,	. 1111	235	5				240	•			
			242																	
	55	(2)	INFC	RMAI	NOI	FOR	SEQ	ID 1	7O:66	5:										

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-	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 253 amino acids(B) TYPE: Amino Acid	1	
5	(D) TOPOLOGY: Linear (xi) SEQUENCE DESCRIPTION: SEQ ID	NO:66:	
	Met Lys Lys Asn Ile Ala Phe Leu Le 1 5		Phe 15
10	Ser Ile Ala Thr Asn Ala Tyr Ala Gl 20	u Val Gln Leu Val Gln s 25	Ser 30
15	Gly Gly Gly Leu Val Gln Pro Gly Gl	y Ser Leu Arg Leu Ser (Cys 45
	Ala Ala Ser Gly Tyr Ser Phe Ser Se 50	er His Tyr Met His Trp ' 55	Val 60
20	Arg Gln Ala Pro Gly Lys Gly Leu Gl 65	u Trp Val Gly Tyr Ile 7	Asp 75
25	Pro Ser Asn Gly Glu Thr Thr Tyr As	sn Gln Lys Phe Lys Gly 85	Arg 90
23	Phe Thr Leu Ser Arg Asp Asn Ser Ly 95		Gln 105
30	Met Asn Ser Leu Arg Ala Glu Asp Th 110	115	120
	Arg Gly Asp Tyr Arg Tyr Asn Gly As 125	130	135
35	Gly Gln Gly Thr Leu Val Thr Val So 140	145	150
40	Pro Ser Val Phe Pro Leu Ala Pro So 155	er Ser Lys Ser Thr Ser 160	GIY 165
	Gly Thr Ala Ala Leu Gly Cys Leu V 170	al Lys Asp Tyr Phe Pro 175	Glu 180
45	Pro Val Thr Val Ser Trp Asn Ser G 185	ly Ala Leu Thr Ser Gly 190	Val 195
	His Thr Phe Pro Ala Val Leu Gln S 200	er Ser Gly Leu Tyr Ser 205	Leu 210
50	Ser Ser Val Val Thr Val Pro Ser S 215	er Ser Leu Gly Thr Gln 220	Thr 225
55	Tyr Ile Cys Asn Val Asn His Lys P 230	235	Asp 240
	Lys Lys Val Glu Pro Lys Ser Cys A	sp Lys Thr His Thr	

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245

250 253

•		(2) INFORMATION FOR SEQ ID NO:67:	
	5	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 159 amino acids (B) TYPE: Amino Acid (D) TOPOLOGY: Linear	
	10	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:	
		Ser Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr Glu Lys Met 1 5 10 15	
	15	Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp Glu Asn 20 25 30	
		Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr 35 40 45	
	20	Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly 50 55 60	
	25	Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Ser 65 70 75	
		Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu 80 85 90	
	30	Met Asn Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val 95 100 105	
		Glu Cys Arg Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu Phe 110 115 120	
	35	Ser Ile Asp Cys Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala 125 130 135	
	40	Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe 140 145 150	
		Ala Asn Ile Leu Arg Asn Lys Glu Ser 155 159	
	45	(2) INFORMATION FOR SEQ ID NO:68:	
	50	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 780 base pairs (B) TYPE: Nucleic Acid (C) STRANDEDNESS: Single (D) TOPOLOGY: Linear	

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

ATGAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT 50

	TG	TACAA	AC G	CATA	CGCTG	TATA	CCAGA	r GAC	CCAGI	rcc (CCGAG	CTCC	C 1	00			
5		CCGCC	TC T	GTGG	GCGAT	AGGG	TCACC	A TCA	CCTG	CAG (STCAP	GTCA	A 15	50		 	
		TTAGT	AC A	TGGT	ATAGG	TAAC	ACGTA	ATT T	CACTO	GT I	ATCAF	CAGA	A 20	00			
	AC	CAGGAA	AA G	CTCC	GAAAC	TACT	GATTT	A CAA	AGTAT	rcc i	AATC	ATTO	T 29	50			
10) CT	GAGTC	CC T	TCTC	GCTTC	TCTG	GATCC	GTT	CTGG	GAC (GGATI	TCAC	T 30	00			•
	CT	ACCAT	'CA G	CAGT	CTGCA	GCCA	GAAGA	C TTC	GCAA	CTT 2	ATTA	TGTI	rc 3!	50			
,		AGAGTA	CT C	ATGT	CCCGC	TCAC	GTTTG	G ACA	.GGGT	ACC .	AAGGT	rggac	SA 4	00			
1:		AAACGA	AC I	GTGG	CTGCA	CCAT	CTGTC	r TCA	TCTT	ccc (GCCAT	rctg <i>i</i>	AT 4	50			
	GA	CAGTI	GA A	ATCT	GGAAC	TGCI	TCTGT	r gtg	TGCC	TGC	TGAA!	raaci	T 5	00			
20) CT	ATCCCA	GA G	AGGC	CAAAG	TACA	GTGGA	A GGT	GGAT	AAC	GCCC:	rcca <i>i</i>	AT 5	50			
	CG	GTAAC	TC C	CAGG	AGAGT	GTC	CAGAG	C AGG	ACAG	CAA	GGAC	AGCA	CC 6	00			
. 2		CAGCCI	CA G	CAGC	ACCCT	GAC	CTGAG	C AAA	GCAG	ACT	ACGA	AAAE	CA 6	50			
2		AAGTCI	CAC G	CCTG	CGAAG	TCAC	CCATC	A GGG	CCTG	AGC	TCGC	CCGT	CA 7	00			
	CA	AAGAGO	TT C	CAACA	.GGGGA	GAG	CGTTAA	G CTG	SATCC	TCT	ACGC	CGGA	CG 7	50			
3	0 CA	rcetee	SCC C	TAGT	'ACGCA	ACT	GTCGT	A 780)								
	(2)	INFOR	ITAMS	ON F	OR SE	Q ID	NO:69	:									
3	5	-) LE	ENGTH	: 242	amin	no aci		,						-		
3	5	(<i>I</i>	1) LE 3) TY	ENGTH PE:		amin Acid	no aci 1										
	((<i>I</i> (I	1) LE 3) TY 0) TO	ENGTH (PE : OPOLO	: 242 Amino GY: I	amin Acio inea	no aci 1	ds	10 : 69	·:							
	(0	(<i>I</i> (E (I xi) SI	A) LE B) TY D) TO EQUEN	ENGTH (PE: OPOLO	ESCRI	amin Acio Linear	no aci i c	ds ID 1			Met	Phe	Val	Phe 15			
	(0 . . Me	(<i>I</i> (I (I xi) SI t Lys	Lys	ENGTH (PE: OPOLO NCE D Asn	Amino GY: I DESCRI	amin Acid inean PTION	no aci i : N: SEQ	ds ID 1	Ala 10	Ser				15			
4	(0 . . Me	(<i>I</i> (I (I xi) SI t Lys	Lys	ENGTH (PE: OPOLO NCE D Asn	Amino GY: I DESCRI	amin Acid inean PTION	no aci i r N: SEQ	ds ID 1	Ala 10	Ser				15			
4	(0 Me Se 5	() (I (I xi) SI t Lys 1	A) LE B) TY C) TO EQUEN Lys Ala	ENGTH (PE: DPOLO NCE D Asn	Asn A	amin Acio inean PTION la PI	no aci i : N: SEQ	ds ID 1 Leu Asp	Ala 10 Ile 25	Ser	Met	Thr	Gln	15 Ser 30			
4	0 Me Se 5	() (I (I xi) SI t Lys 1 r Ile	Lys Ala Ser	POPOLO Asn Thr	I: 242 Amino OGY: I DESCRI Ile # 5 Asn # 20 Ser # 35	amin Acid inear PTION Ala Pl	no aci i n: SEQ ne Leu yr Ala	ds ID 1 Leu Asp	Ala 10 Ile 25 Asp 40	Ser Gln Arg	Met Val	Thr	Gln 	15 Ser 30 Thr 45			
4	0 Me Se 5 Pr	(A) (I) (I) (I) (I) (I) (I) (I) (I) (I) (I	Lys Ala Ser	POPOLO Asn Thr Leu Ser	I: 242 Amino OGY: I DESCRI Ile # 5 Asn # 20 Ser # 35 Gln S	amin Acid inear PTION Ala P	no aci	ds ID 1 Leu Asp Gly	Ala 10 Ile 25 Asp 40 Gly 55	Ser Gln Arg	Met Val Gly	Thr Thr Asn	Gln Ile Thr	15 Ser 30 Thr 45 Tyr 60			
4	0 Me Se 5 Pr	(A) (I) (I) (I) (I) (I) (I) (I) (I) (I) (I	Lys Ala Ser	POPOLO Asn Thr Leu Ser	I: 242 Amino OGY: I DESCRI Ile # 5 Asn # 20 Ser # 35 Gln S	amin Acid inear PTION Ala P	no aci i n: SEQ ne Leu yr Ala	ds ID 1 Leu Asp Gly	Ala 10 Ile 25 Asp 40 Gly 55	Ser Gln Arg	Met Val Gly	Thr Thr Asn	Gln Ile Thr	15 Ser 30 Thr 45 Tyr 60			

	8	0	85	90
•	-Ser-Gly-Ser-Gly-Se	r Gly Thr Asp Phe	Thr Leu Thr Ile Ser	Ser
	9		100	105
5	Leu Gln Pro Glu As	p Phe Ala Thr Tyr	Tyr Cys Ser Gln Ser	Thr
	11		115	120
	vic Val Pro Leu Th	r Phe Glv Gln Gly	Thr Lys Val Glu Ile	e Lys
10	12		130	135
	Now what Wal Ala Al	a Pro Ser Val Phe	Ile Phe Pro Pro Se	r Asp
	Arg IIII var Ara Ar		145	150
	-1 - d1 - Y True Co	w Clar The Ala Ser	Val Val Cys Leu Le	u Asn
15	Giu Gin Leu Lys Se		160	165
		et - 11 - Ton 11-1	al- T- Iva Val As	n Aen
	Asn Phe Tyr Pro Ar		Gln Trp Lys Val As 175	180
20			a trail mbu din di	
	Ala Leu Gln Ser Gl 18		Ser Val Thr Glu Gl 190	195
	•			
26	Ser Lys Asp Ser Th		Ser Thr Leu Thr Le 205	u ser 210
25				
	Lys Ala Asp Tyr Gl		Tyr Ala Cys Glu Va	1 Thr 225
30			Lys Ser Phe Asn Ai	rg Gly 240
	23	30	233	
	Glu Cys			
35	242			
33	(2) INFORMATION FOR	R SEQ ID NO:70:		
	(i) SEQUENCE CH	ARACTERISTICS:		
	(A) LENGTH:	253 amino acids		
40	(B) TYPE: As (D) TOPOLOG			
	(xi) SEQUENCE DE	SCRIPTION: SEQ ID	NO:70:	
45	Met Lys Lys Asn I	le Ala Phe Leu Leu	a Ala Ser Met Phe V	al Phe
	1	5	10	15
	Ser Ile Ala Thr A	sn Ala Tyr Ala Glu	ı Val Gln Leu Val G	lu Ser
		20	25	30
50	Gly Gly Gly Leu V	al Gln Pro Glv Glv	y Ser Leu Arg Leu S	er Cys
	_	35	40	45
	N1= N1= Co~ Cl·· m	hm Car Dha Car Ca	r His Tyr Met His 1	rp Val
55	Ata Ata Ser Giy T	50	55	60
	•			

		Lys	Gln	Ala	Pro	Gly 65	Lys	Gly	Leu	Glu	Trp 70	Val	Gly	Tyr	Ile	Asp 75			
19	5	Pro	Ser	Asn	Gly	Glu 80	Thr	Thr	Tyr	Asn	Gln 85	Lys	Phe	Lys	Gly	Arg 90		1-1-2-1	
		Phe	Thr	Leu	Ser	Arg 95	Asp	Asn	Ser	Lys	Asn 100	Thr	Ala	Tyr	Leu	Gln 105			
1	0	Met	Asn	Ser	Leu	Arg 110	Ala	Glu	Asp	Thr	Ala 115	Val	Tyr	Tyr	Cys	Ala 120			
	5	Arg	Gly	Asp	Tyr	Arg 125	Tyr	Asn	Gly	Asp	Trp 130	Phe	Phe	Asp	Val	Trp 135			
1	.5	Gly	Gln	Gly	Thr	Leu 140	Val	Thr	Val	Ser	Ser 145	Ala	Ser	Thr	Lys	Gly 150			
2	20	Pro	Ser	Val	Phe	Pro 155	Leu	Ala	Pro	Ser	Ser 160	Lys	Ser	Thr	Ser	Gly 165	 		
		Gly	Thr	Ala	Ala	Leu 170	Gly	Cys	Leu	Val	Lys 175	Asp	Tyr	Phe	Pro	Glu 180			
2	25			Thr		185					190					195			
5	30	•		Phe		200					205					210			
				Val		215					220					225			
3	35	-		Cys		230					235					Asp 240			
		-	_	Val		245					Lys 250		His	Thr 253					
•	40		i) S	RMAT:	NCE (CHAR	ACTE	RIST	ics:					-					
	45		(A) Li B) T D) T	YPE:	Ami	no A	cid	acı	as									
		-		EQUE											•••	D).			
	50	1				5					10)				15			
						20				·	25					ser 30			
	55	Pro	Ser	Ser	Leu	Ser 35		Ser	· Val	. Gly	Asp 40		y Val	L Th	r Il	Thr 45			

-	Cys	Arg	Ser	Ser	Gln 5.0_	Ser	Leu	Val	His	Gly 55	Ile	Gly	Ala	Thr	Tyr 60		 	
5	Leu	His	Trp	Tyr	Gln 65	Gln	Lys	Pro	Gly	Lys 70	Ala	Pro	Lys	Leu	Leu 75			
	Ile	Tyr	Lys	Val	Ser 80	Asn	Arg	Phe	Ser	Gly 85	Val	Pro	Ser	Arg	Phe 90			
10	Ser	Gly	Ser	Gly	Ser 95	Gly	Thr	Asp	Phe	Thr 100	Leu	Thr	Ile	Ser	Ser 105	•		
15	Leu	Gln	Pro	Glu	Asp 110	Phe	Ala	Thr	Tyr	Tyr 115	Cys	Ser	Gln	Ser	Thr 120			
	His	Val	Pro	Leu	Thr 125	Phe	Gly	Gln	Gly	Thr 130	Lys	Val	Glu	Ile	Lys 135	÷		
20	Arg	Thr	Val	Ala	Ala 140		Ser	Val	Phe	Ile 145		Pro	Pro	Ser	Asp 150	<u>-</u>	 	
	Glu	Gln	Leu	Lys	Ser 155	Gly	Thr	Ala	Ser	Val 160	Val	Cys	Leu	Leu	Asn 165			
25	Asn	Phe	Tyr	Pro	Arg 170		Ala	Lys	Val	Gln 175		Lys	Val	Asp	Asn 180			
30	Ala	Leu	Gln	Ser	Gly 185		Ser	Gln	. Glu	Ser 190		Thr	Glu	Gln	195			
	Ser	Lys	Asp	Ser	Thr 200		Ser	Leu	Ser	Ser 205	Thr	Lev	Thr	Leu	210	•	-	
35					215	;				220)				225			
40	His	Gln	Gly	Leu	230		Pro	val	Thi	235	s Sei	r Phe	e Ası	a Arg	240			
40	Glu	Cys 242																
45	(2)	INFO	RMAT	NOI	FOR	SEQ	ID 1	10 : 72	2:									
	(((A)		TH: 4	15 ar	nino	acio	_									
50				OPOI														
50								: SE					_			·		
£		s Pro	Pro	Cys		o Ala	a Pro	o Gl	u Le	u Le 1		y Gl	y Ar	g Me	t Lys 15			
55	Glr	n Let	ı Glı	ı Asp	p Ly	s Va	l Gl	u Gl	u Le	u Le	u Se	r Ly	s As	n Ty	r His			

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20 25 ,30

Leu Glu Asn Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg
35 40 45

5

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- (2) INFORMATION FOR SEQ ID NO:73:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 780 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

15

30

TGCTACAAAC GCATACGCTG ATATCCAGAT GACCCAGTCC CCGAGCTCCC 100

ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT 50

AGCTTAGTAC ATGGTATAGG TGCTACGTAT TTACACTGGT ATCAACAGAA 200

25 ACCAGGAAAA GCTCCGAAAC TACTGATTTA CAAAGTATCC AATCGATTCT 250

CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC GGATTTCACT 300

CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTCGCAACTT ATTACTGTTC 350

ACAGAGTACT CATGTCCCGC TCACGTTTGG ACAGGGTACC AAGGTGGAGA 400

TCAAACGAAC TGTGGCTGCA CCATCTGTCT TCATCTTCCC GCCATCTGAT 450

35 GAGCAGTTGA AATCTGGAAC TGCTTCTGTT GTGTGCCTGC TGAATAACTT 500

CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC GCCCTCCAAT 550

CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC 600

TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA 650

CAAAGTCTAC GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA 700

45 CAAAGAGCTT CAACAGGGGA GAGTGTTAAG CTGATCCTCT ACGCCGGACG 750

CATCGTGGCC CTAGTACGCA ACTAGTCGTA 780

(2) INFORMATION FOR SEQ ID NO:74:

50

MICHOCID -MIC GR3720042 1 -

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 927 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
- 55 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

	AAAAGGGTAT CTAGAGGTTG AGGTGATTTT ATGAAAAAGA ATATCGCATT 50
. 5	TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC GCGTACGCTG 100
	AGGTTCAGCT AGTGCAGTCT GGCGGTGGCC TGGTGCAGCC AGGGGGCTCA 150
10	CTCCGTTTGT CCTGTGCAGC TTCTGGCTAC TCCTTCTCGA GTCACTATAT 200
	GCACTGGGTC CGTCAGGCCC CGGGTAAGGG CCTGGAATGG GTTGGATATA 250
	TTGATCCTTC CAATGGTGAA ACTACGTATA ATCAAAAGTT CAAGGGCCGT 300
15	TTCACTTTAT CTCGCGACAA CTCCAAAAAC ACAGCATACC TGCAGATGAA 350
	CAGCCTGCGT GCTGAGGACA CTGCCGTCTA TTACTGTGCA AGAGGGGATT 400
20	ATCGCTACAA TGGTGACTGG TTCTTCGACG TCTGGGGTCA AGGAACCCTG 450
	GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC 500
	ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG 550
25	TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC 600
	CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT 650
30	CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC 700
	AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTCGAC 750
25	AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCGCCGTG 800
35	CCCAGCACCA GAACTGCTGG GCGGCCGCAT GAAACAGCTA GAGGACAAGG 850
	TCGAAGAGCT ACTCTCCAAG AACTACCACC TAGAGAATGA AGTGGCAAGA 900
40	CTCAAAAAGC TTGTCGGGGA GCGCTAA 927
	(2) INFORMATION FOR SEQ ID NO:75:
45	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 298 amino acids
	(B) TYPE: Amino Acid (D) TOPOLOGY: Linear
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:
50	Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe 1 5 10 15
	Ser Ile Ala Thr Asn Ala Tyr Ala Glu Val Gln Leu Val Gln Ser
55	20 25 30

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		Gly	Gly	Gly	Leu	Val 35	Gln	Pro	Gly	Gly	Ser 40	Leu	Arg	Leu	Ser,	Cys 45
	5	Ala	Ala	Ser	Gly	Tyr 50	Ser	Phe	Ser	Ser	His 55	Tyr	Met	His	Trp	Val 60
		Arg	Gln	Ala	Pro	Gly 65	Lys	Gly	Leu	Glu	Trp 70	Val	Gly	Tyr	Ile	Asp 75
1	0	Pro	Ser	Asn	Gly	Glu 80	Thr	Thr	Tyr	Asn	Gln 85	Lys	Phe	Lys	Gly	Arg 90
1	5	Phe	Thr	Leu	Ser	Arg 95	Asp	Asn	Ser	Lys	Asn 100	Thr	Ala	Tyr	Leu	Gln 105
•	J	Met	Asn	Ser	Leu	Arg 110	Ala	Glu	Asp	Thr	Ala 115	Val	Tyr	Tyr	Cys	Ala 120
2	0	Arg	Gly	Asp	Tyr	Arg 125	Tyr	Asn	Gly	Asp	Trp 130	Phe	Phe	Asp	Val	Trp 135
		Gly	Gln	Gly	Thr	Leu 140	Val	Thr	Val	Ser	Ser 145	Ala	Ser	Thr	Lys	Gly 150
2	5	Pro	Ser	Val	Phe	Pro 155	Leu	Ala	Pro	Ser	Ser 160	Lys	Ser	Thr	Ser	Gly 165
3	0	Gly	Thr	Ala	Ala	Leu 170	Gly	Cys	Leu	Val	Lys 175	Asp	Tyr	Phe	Pro	Glu 180
		Pro	Val	Thr	Val	Ser 185	Trp	Asn	Ser	Gly	Ala 190	Leu	Thr	Ser	Gly	Val 195
3	5	His	Thr	Phe	Pro	Ala 200	Val	Leu	Gln	Ser	Ser 205	Gly	Leu	Tyr	Ser	Leu 210
		Ser	Ser	Val	Val	Thr 215	Val	Pro	Ser	Ser	Ser 220	Leu	Gly	Thr	Gln	Thr 225
4	10	Tyr	Ile	Cys	Asn	Val 230	Asn	His	Lys	Pro	Ser 235	Asn	Thr	Lys	Val	Asp 240
4	15	Lys	Lys	Val	Glu	Pro 245	Lys	Ser	Cys	Asp	Lys 250	Thr	His	Thr	Сув	Pro 255
		Pro	Cys	Pro	Ala	Pro 260	Glu	Leu	Leu	Gly	Cly 265	Arg	Met	Lys	Gln	Leu 270
5	5 0	Glu	Asp	Lys	Val	Glu 275	Glu	Leu	Leu	Ser	Lys 280	Asn	Tyr	His	Leu	Glu 285
		Asn	Glu	Val	Ala	Arg 290	Leu	Lys	Lys	Leu	Val 295	Gly	Glu	Arg 298		
5	55	(2)	INFOF	ITAMS	ON F	FOR S	SEQ 1	D NO	76:76	:						

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6563 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
- 5 (D) TOPOLOGY: Linear
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

10	GAATTCAACT	TCTCCATACT	TTGGATAAGG	AAATACAGAC	ATGAAAAATC	50
	TCATTGCTGA (GTTGTTATTT	AAGCTTGCCC	AAAAAGAAGA	AGAGTCGAAT	100
	GAACTGTGTG	CGCAGGTAGA	AGCTTTGGAG	ATTATCGTCA	CTGCAATGCT	150
15	TCGCAATATG	GCGCAAAATG	ACCAACAGCG	GTTGATTGAT	CAGGTAGAGG	200
	GGGCGCTGTA	CGAGGTAAAG	CCCGATGCCA	GCATTCCTGA	CGACGATACG	250
20	GAGCTGCTGC	GCGATTACGT	AAAGAAGTTA	TTGAAGCATC	CTCGTCAGTA	300
 	AAAAGTTAAT	CTTTTCAACA	GCTGTCATAA	AGTTGTCACG	GCCGAGACTT	350
	ATAGTCGCTT	TGTTTTTATT	TTTTAATGTA	TTTGTAACTA	GAATTCGAGC	400
25	TCGGTACCCG	GGGATCCTCT	CGAGGTTGAG	GTGATTTTAT	GAAAAAGAAT	450
	ATCGCATTTC	TTCTTGCATC	TATGTTCGTT	TTTTCTATTG	CTACAAACGC	500
30	ATACGCTGAT	ATCCAGATGA	CCCAGTCCCC	GAGCTCCCTG	TCCGCCTCTG	550
•	TGGGCGATAG	GGTCACCATC	ACCTGCAGGT	CAAGTCAAAG	CTTAGTACAT	600
25	GGTATAGGTG	CTACGTATTT	ACACTGGTAT	CAACAGAAAC	CAGGAAAAGC	650
35	TCCGAAACTA	CTGATTTACA	AAGTATCCAA	TCGATTCTC1	GGAGTCCCTT	700
	CTCGCTTCTC	TGGATCCGGT	TCTGGGACGG	ATTTCACTC	GACCATCAGC	750
40	AGTCTGCAGC	CAGAAGACTT	CGCAACTTAT	TACTGTTCAC	: AGAGTACTCA	800
	TGTCCCGCTC	ACGTTTGGAC	: AGGGTACCA	A GGTGGAGAT	CAAACGAACTG	850
45	TGGCTGCACC	ATCTGTCTTC	ATCTTCCCG	CATCTGATG	A GCAGTTGAAA	.900
 45	TCTGGAACTG	CTTCTGTTGT	CTGCCTGCT(S AATAACTTC	r atcccagagi	950
	GGCCAAAGTA	CAGTGGAAGG	TGGATAACG	C CCTCCAATC	G GGTAACTCC	2 1000
50	AGGAGAGTGT	CACAGAGCAC	GACAGCAAG	G ACAGCACCT	A CAGCCTCAG	2 1050
	AGCACCCTGA	CGCTGAGCA	A AGCAGACTA	c gagaaacac	A AAGTCTACG	C 1100
	CTGCGAAGTC	ACCCATCAGO	G GCCTGAGCT	C GCCCGTCAC	A AAGAGCTTC	A 1150
55	ACAGGGGAGA	GTGTTAAGC'	r gatcctcta	C GCCGGACGC	A TCGTGGCCC	T 1200

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	AGTACGCAAC	TAGTCGTAAA	AAGGGTATCT	AGAGGTTGAG	GTGATTTTAT	1250
	GAAAAAGAAT	ATCGCATTTC	TTCTTGCATC	TATGTTCGTT	TTTTCTATTG	1300
5	CTACAAACGC	GTACGCTGAG	GTTCAGCTAG	TGCAGTCTGG	CGGTGGCCTG	1350
	GTGCAGCCAG	GGGGCTCACT	CCGTTTGTCC	TGTGCAGCTT	CTGGCTACTC	1400
10	CTTCTCGAGT	CACTATATGC	ACTGGGTCCG	TCAGGCCCCG	GGTAAGGGCC	1450
	TGGAATGGGT	TGGATATATT	GATCCTTCCA	ATGGTGAAAC	TACGTATAAT	1500
15	CAAAAGTTCA	AGGGCCGTTT	CACTTTATCT	CGCGACAACT	CCAAAAACAC	1550
15	AGCATACCTG	CAGATGAACA	GCCTGCGTGC	TGAGGACACT	GCCGTCTATT	1600
	ACTGTGCAAG	AGGGGATTAT	CGCTACAATG	GTGACTGGTT	CTTCGACGTC	1650
20	TGGGGTCAAG	GAACCCTGGT	CACCGTCTCC	TCGGCCTCCA	CCAAGGGCCC	1700
	ATCGGTCTTC	CCCCTGGCAC	CCTCCTCCAA	GAGCACCTCT	GGGGCACAG	1750
25	CGGCCCTGGG	CTGCCTGGTC	AAGGACTACT	TCCCCGAACC	GGTGACGGTG	1800
23	TCGTGGAACT	CAGGCGCCCT	GACCAGCGGC	GTGCACACCT	TCCCGGCTGT	1850
	CCTACAGTCC	TCAGGACTCT	ACTCCCTCAG	CAGCGTGGTG	ACCGTGCCCT	1900
30	CCAGCAGCTT	GGGCACCCAG	ACCTACATCT	GCAACGTGAA	TCACAAGCCC	1950
	AGCAACACCA	AGGTCGACAA	GAAAGTTGAG	CCCAAATCTT	GTGACAAAAC	2000
35	TCACACATGC	CCGCCGTGCC	CAGCACCAGA	ACTGCTGGGC	GGCCGCATGA	2050
35	AACAGCTAGA	GGACAAGGTC	GAAGAGCTAC	TCTCCAAGAA	CTACCACCTA	2100
	GAGAATGAAG	TGGCAAGACT	CAAAAAGCTT	GTCGGGGAGC	GCTAAGCATG	2150
40	CGACGGCCCT	AGAGTCCCTA	ACGCTCGGTT	GCCGCCGGGC	GTTTTTTATT	2200
	GTTAACTCAT	GTTTGACAGC	TTATCATCGA	TAAGCTTTAA	TGCGGTAGTT	2250
45	TATCACAGTT	AAATTGCTAA	CGCAGTCAGG	CACCGTGTAT	GAAATCTAAC	2300
	AATGCGCTCA	TCGTCATCCT	CGGCACCGTC	ACCCTGGATG	CTGTAGGCAT	2350
	AGGCTTGGTT	ATGCCGGTAC	TGCCGGGCCT	CTTGCGGGAT	ATCGTCCATT	2400
50	CCGACAGCAT	CGCCAGTCAC	TATGGCGTGC	TGCTAGCGCT	ATATGCGTTG	2450
	ATGCAATTTC	TATGCGCACC	CGTTCTCGGA	GCACTGTCCG	ACCGCTTTGG	2500
55	CCGCCGCCCA	GTCCTGCTCG	CTTCGCTACT	TGGAGCCACT	· ATCGACTACG	2550
	CGATCATGGC	GACCACACCC	GTCCTGTGGA	TCCTCTACGO	CGGACGCATC	2600

	GTGGCCGGCA	TCACCGGCGC	CACAGGTGCG	GTTGCTGGCG	CCTATATCGC	2650
	CGACATCACC	GATGGGGAAG	ATCGGGCTCG	CCACTTCGGG	CTCATGAGCG	2700
5	CTTGTTTCGG	CGTGGGTATG	GTGGCAGGCC	CCGTGGCCGG	GGGACTGTTG	2750
	GGCGCCATCT	CCTTGCACGC	ACCATTCCTT	GCGGCGGCGG	TGCTCAACGG	2800
10	CCTCAACCTA	CTACTGGGCT	GCTTCCTAAT	GCAGGAGTCG	CATAAGGGAG	2850
	AGCGTCGTCC	GATGCCCTTG	AGAGCCTTCA	ACCCAGTCAG	CTCCTTCCGG	2900
	TGGGCGCGGG	GCATGACTAT	CGTCGCCGCA	CTTATGACTG	TCTTCTTTAT	2950
15	CATGCAACTC	GTAGGACAGG	TGCCGGCAGC	GCTCTGGGTC	ATTTTCGGCG	3000
	AGGACCGCTT	TCGCTGGAGC	GCGACGATGA	TCGGCCTGTC	GCTTGCGGTA	3050
20	TTCGGAATCT	TGCACGCCCT	CGCTCAAGCC	TTCGTCACTG	GTCCCGCCAC	3100
	CAAACGTTTC	GGCGAGAAGC	AGGCCATTAT	CGCCGGCATG	GCGGCCGACG	3150
	CGCTGGGCTA	CGTCTTGCTG	GCGTTCGCGA	CGCGAGGCTG	GATGGCCTTC	3200
25	CCCATTATGA	TTCTTCTCGC	TTCCGGCGGC	ATCGGGATGC	CCGCGTTGCA	3250
	GGCCATGCTG	TCCAGGCAGG	TAGATGACGA	CCATCAGGGA	CAGCTTCAAG	3300
30	GATCGCTCGC	GGCTCTTACC	AGCCTAACTT	CGATCACTGG	ACCGCTGATO	3350
	GTCACGGCGA	TTTATGCCGC	CTCGGCGAGC	ACATGGAACG	GGTTGGCAT	3400
	GATTGTAGGC	GCCGCCCTAT	ACCTTGTCTG	CCTCCCCGC	TTGCGTCGCC	3 3450
35	GTGCATGGAG	CCGGGCCACC	TCGACCTGAA	TGGAAGCCG	GGGCACCTCC	3 3500
	CTAACGGATT	CACCACTCCA	AGAATTGGAG	CCAATCAAT	r cttgcggagi	A 3550
40	ACTGTGAATG	CGCAAACCAA	CCCTTGGCAG	AACATATCC	A TCGCGTCCG	C 3600
	CATCTCCAGC	AGCCGCACGC	GGCGCATCTC	GGGCAGCGT	T GGGTCCTGG	C 3650
	CACGGGTGCG	CATGATCGT	CTCCTGTCG	TGAGGACCC	G GCTAGGCTG	G 3700
45	CGGGGTTGCC	TTACTGGTT	GCAGAATGA	TCACCGATA	C-GCGAGCGAA	C 3750
	GTGAAGCGAC	TGCTGCTGC	AAACGTCTG	GACCTGAGC	A ACAACATGA	A 3800
50	TGGTCTTCGG	TTTCCGTGT	TCGTAAAGT	C TGGAAACGC	G GAAGTCAGC	G 3850
	CCCTGCACC	A TTATGTTCC	GATCTGCAT	C GCAGGATGC	T GCTGGCTAC	C 3900
	CTGTGGAAC	A CCTACATCT	G TATTAACGA	A GCGCTGGCA	T TGACCCTGA	AG 3950
55	TGATTTTTC	r CTGGTCCCG	C CGCATCCAT	A CCGCCAGTI	G TTTACCCTC	A 4000

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							1
•		CAACGTTCCA	GTAACCGGGC	ATGTTCATCA	TCAGTAACCC	GTATCGTGAG	4050
	5	CATCCTCTCT	CGTTTCATCG	GTATCATTAC	CCCCATGAAC	AGAAATTCCC	4100
	3	CCTTACACGG	AGGCATCAAG	TGACCAAACA	GGAAAAAACC	GCCCTTAACA	4150
		TGGCCCGCTT	TATCAGAAGC	CAGACATTAA	CGCTTCTGGA	GAAACTCAAC	4200
	10 .	GAGCTGGACG	CGGATGAACA	GGCAGACATC	TGTGAATCGC	TTCACGACCA	4250
		CGCTGATGAG	CTTTACCGCA	GCTGCCTCGC	GCGTTTCGGT	GATGACGGTG	4300
	16	AAAACCTCTG	ACACATGCAG	CTCCCGGAGA	CGGTCACAGC	TTGTCTGTAA	4350
	15	GCGGATGCCG	GGAGCAGACA	AGCCCGTCAG	GGCGCGTCAG	CGGGTGTTGG	4400
		CGGGTGTCGG	GGCGCAGCCA	TGACCCAGTC	ACGTAGCGAT	AGCGGAGTGT	4450
	20	ATACTGGCTT	AACTATGCGG	CATCAGAGCA	GATTGTACTG	AGAGTGCACC	4500
		ATATGCGGTG	TGAAATACCG	CACAGATGCG	TAAGGAGAAA	ATACCGCATC	4550
	25	AGGCGCTCTT	CCGCTTCCTC	GCTCACTGAC	TCGCTGCGCT	CGGTCGTTCG	4600
	23	GCTGCGGCGA	GCGGTATCAG	CTCACTCAAA	GGCGGTAATA	CGGTTATCCA	4650
		CAGAATCAGG	GGATAACGCA	GGAAAGAACA	TGTGAGCAAA	AGGCCAGCAA	4700
	30	AAGGCCAGGA	ACCGTAAAAA	GGCCGCGTTG	CTGGCGTTTT	TCCATAGGCT	4750
		CCGCCCCCT	GACGAGCATC	ACAAAAATCG	ACGCTCAAGT	CAGAGGTGGC	4800
	35	GAAACCCGAC	AGGACTATAA	AGATACCAGG	CGTTTCCCCC	TGGAAGCTCC	4850
	•	CTCGTGCGCT	CTCCTGTTCC	GACCCTGCCG	CTTACCGGAT	ACCTGTCCGC	4900
		CTTTCTCCCT	TCGGGAAGCG	TGGCGCTTTC	TCATAGCTCA	CGCTGTAGGT	4950
	40	ATCTCAGTTC	GGTGTAGGTC	GTTCGCTCCA	AGCTGGGCTG	TGTGCACGAA	5000
		CCCCCGTTC	AGCCCGACCG	CTGCGCCTTA	TCCGGTAACT	ATCGTCTTGA	5050
	45	GTCCAACCCG	GTAAGACACG	ACTTATCGCC	ACTGGCAGCA	GCCACTGGTA	5100
		ACAGGATTAG	CAGAGCGAGG	TATGTAGGCG	GTGCTACAGA	GTTCTTGAAG	-5150
		TGGTGGCCTA	ACTACGGCTA	CACTAGAAGG	ACAGTATTTG	GTATCTGCGC	5200
	50	TCTGCTGAAG	CCAGTTACCT	TCGGAAAAAG	AGTTGGTAGC	TCTTGATCCG	5250
		GCAAACAAAC	CACCGCTGGT	AGCGGTGGTT	TTTTTGTTTG	CAAGCAGCAG	5300
	55	ATTACGCGCA	GAAAAAAAGG	ATCTCAAGAA	GATCCTTTGA	TCTTTTCTAC	5350
	در	GGGGTCTGAC	GCTCAGTGGA	ACGAAAACTC	ACGTTAAGGG	ATTTTGGTCA	5400

	TGAGATTATC	AAAAAGGATC	TTCACCTAGA	TCCTTTTAAA	TTAAAAATGA	5450
	AGTTTTAAAT	CAATCTAAAG	TATATATGAG	TAAACTTGGT	CTGACAGTTA	5500
5	CCAATGCTTA	ATCAGTGAGG	CACCTATCTC	AGCGATCTGT	CTATTTCGTT	5550
	CATCCATAGT	TGCCTGACTC	CCCGTCGTGT	AGATAACTAC	GATACGGGAG	5600
10	GGCTTACCAT	CTGGCCCCAG	TGCTGCAATG	ATACCGCGAG	ACCCACGCTC	5650
	ACCGGCTCCA	GATTTATCAG	CAATAAACCA	GCCAGCCGGA	AGGGCCGAGC	5700
	GCAGAAGTGG	TCCTGCAACT	TTATCCGCCT	CCATCCAGTC	TATTAATTGT	5750
15	TGCCGGGAAG	CTAGAGTAAG	TAGTTCGCCA	GTTAATAGTT	TGCGCAACGT	5800
	TGTTGCCATT	GCTGCAGGCA	TCGTGGTGTC	ACGCTCGTCG	TTTGGTATGG	5850
20	CTTCATTCAG	CTCCGGTTCC	CAACGATCAA	GGCGAGTTAC	ATGATCCCCC	5900
	ATGTTGTGCA	AAAAAGCGGT	TAGCTCCTTC	GGTCCTCCGA	TCGTTGTCAG	5950
0.5	AAGTAAGTTG	GCCGCAGTGT	TATCACTCAT	GGTTATGGCA	GCACTGCATA	4 6000
25	ATTCTCTTAC	TGTCATGCCA	TCCGTAAGAT	GCTTTTCTGI	GACTGGTGAG	6 6 6 0 5 0
	TACTCAACCA	AGTCATTCTG	AGAATAGTGT	ATGCGGCGAC	CGAGTTGCT	6100
30	TTGCCCGGCG	TCAACACGGG	ATAATACCGC	GCCACATAGO	AGAACTTTAI	A 6150
	AAGTGCTCAT	CATTGGAAAA	CGTTCTTCGG	GGCGAAAAC	CTCAAGGAT	C 6200
2.5	TTACCGCTGI	TGAGATCCAG	TTCGATGTA	CCCACTCGT	G CACCCAACT	G 6250
35	ATCTTCAGCA	TCTTTTACTT	TCACCAGCGT	TTCTGGGTG	A GCAAAAACA	G 6300
	GAAGGCAAAA	TGCCGCAAAA	AAGGGAATA	A GGGCGACAC	G GAAATGTTG	A 6350
40	ATACTCATAC	TCTTCCTTTT	TCAATATTA	r TGAAGCATT	T ATCAGGGTT	A 6400
	TTGTCTCATO	agcggataca	TATTTGAAT	AADATTTAGAA	A AATAAACAA	A 6450
45	TAGGGGTTC	C GCGCACATTI	CCCCGAAAA	G TGCCACCTG	A CGTCTAAGA	A 6500
 45	ACCATTATT	A TCATGACATT	AACCTATAA	A AATAGGCGT	A TCACGAGGC	CC_6550
						•

CTTTCGTCTT CAA 6563

WE CLAIM:

- 1. A conjugate consisting essentially of one or more antibody fragments covalently attached to one or more nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD.
 - 2. The conjugate of claim 1, wherein the apparent size of the conjugate is at least about 800 kD.
- The conjugate of claim 1, wherein the apparent size of the conjugate is at least about 1,400 kD.
 - 4. The conjugate of claim 1, wherein the apparent size of the conjugate is at least about 1,800 kD.

5. The conjugate of claim 1, wherein the apparent size of the conjugate is at least about 8 fold greater than the apparent size of the antibody fragment.

- 6. The conjugate of claim 5, wherein the apparent size of the conjugate is at least about 15 fold greater than the apparent size of the antibody fragment.
 - 7. The conjugate of claim 6, wherein the apparent size of the conjugate is at least about 25 fold greater than the apparent size of the antibody fragment.
- 25 8. The conjugate of claim 1, wherein the conjugate contains no more than one antibody fragment, and wherein the antibody fragment is selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv and F(ab')₂.
 - 9. The conjugate of claim 8 wherein the antibody fragment is F(ab')₂.

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- 10. The conjugate of claim 1 wherein the antibody fragment is covalently attached to no more than about 10 nonproteinaceous polymer molecules.
- 11. The conjugate of claim 10 wherein the antibody fragment is covalently attached to no more than about 5 nonproteinaceous polymer molecules.

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12. The conjugate of claim 11 wherein the antibody fragment is covalently attached to no more than about 2 nonproteinaceous polymer molecules.

- 13. The conjugate of claim 12 wherein the antibody fragment is attached to no more than 1 nonproteinaceous polymer molecule.
 - 14. The conjugate of claim 12, wherein the antibody fragment comprises a heavy chain and a light chain derived from a parental antibody, wherein in the parental antibody the heavy and light chains are covalently linked by a disulfide bond between a cysteine residue in the light chain and a cysteine residue in the heavy chain, wherein in the antibody fragment the cysteine residue in the light or heavy chain is substituted with another amino acid and the cysteine residue in the opposite chain is covalently linked to a nonproteinaceous polymer molecule.
- 15. The conjugate of claim 8 wherein the antibody fragment is selected from the group consisting of Fab, Fab' and Fab'-SH.
 - 16. The conjugate of claim 15 wherein the antibody fragment is covalently attached to no more than 1 nonproteinaceous polymer molecule.
- 20 17. The conjugate of claim 16 wherein the nonproteinaceous polymer molecule in the conjugate is covalently attached to the hinge region of the antibody fragment.
 - 18. The conjugate of claim 1 wherein the nonproteinaceous polymer is a polyethylene glycol (PEG).
 - 19. The conjugate of claim 18 wherein the PEG has an average molecular weight of at least about 20 kD.
- 20. The conjugate of claim 19 wherein the PEG has an average molecular weight of at least about 40 kD.
 - 21. The conjugate of claim 20 wherein the PEG is a single chain molecule.
 - 22. The conjugate of claim 20 wherein the PEG is a branched chain molecule.

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- 23. The conjugate of claim 19, wherein the conjugate contains no more than one antibody fragment, and wherein the antibody fragment is a F(ab')₂ and is covalently attached to no more than about 2 PEG molecules.
- The conjugate of claim 19, wherein the conjugate contains no more than one antibody fragment, and wherein the antibody fragment is selected from the group consisting of Fab, Fab' and Fab'-SH and is covalently attached to no more than one PEG molecule.
- The conjugate of claim 24 wherein the PEG molecule is covalently attached to the hinge region of the antibody fragment.
 - 26. The conjugate of claim 1 wherein the antibody fragment has an antigen binding site that binds to human IL-8.
- 15 27. The conjugate of claim 26, wherein the conjugate contains no more than one antibody fragment, wherein the antibody fragment is selected from the group consisting of Fab, Fab' and Fab'-SH, wherein the antibody fragment is covalently attached to no more than one nonproteinaceous polymer molecule, and wherein the nonproteinaceous polymer molecule is a polyethylene glycol having an actual molecular weight of at least about 30 kD.

28. The conjugate of claim 1 wherein the antibody fragment is humanized.

- 29. The conjugate of claim 1 wherein the conjugate contains no more than one antibody fragment.
 - 30. A composition comprising the conjugate of claim 1 and a carrier.
 - 31. The composition of claim 30 that is sterile.
- 30. A conjugate formed by one or more antibody fragments covalently attached to one or more nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD, and wherein the molecular structure of the conjugate is free of other matter.
 - 33. A conjugate formed by one or more antibody fragments covalently attached to one or more nonproteinaceous polymer molecules, wherein the apparent size of the conjugate is at least about 500 kD, wherein the antibody fragment incorporates a nonproteinaceous label free of any polymer, and wherein the molecular structure of the conjugate is free of other matter.

34. The conjugate of claim 33 wherein the nonproteinaceous label is a radiolabel.

35. A polypeptide selected from the group consisting of: (1) a polypeptide that is an anti-IL-8 monoclonal antibody or antibody fragment comprising a light chain amino acid sequence comprising the complementarity determining regions of the light chain polypeptide amino acid sequence of Fig. 36; and (2) a polypeptide that is an anti-IL-8 monoclonal antibody or antibody fragment comprising a light chain amino acid sequence comprising the complementarity determining regions of the light chain polypeptide amino acid sequence of Fig. 45.

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- 36. The polypeptide of claim 35, wherein the light chain amino acid sequence comprises the complementarity determining regions of the light chain polypeptide amino acid sequence of Fig. 45.
- The polypeptide of claim 35 that further comprises a heavy chain amino acid sequence comprising the complementarity determining regions of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B.
 - 38. The polypeptide of claim 35 wherein the light chain amino acid sequence is selected from the group consisting of: (1) a light chain amino acid sequence comprising amino acids 1-219 of the light chain polypeptide amino acid sequence of Fig. 36; and (2) a light chain amino acid sequence comprising amino acids 1-219 of the light chain polypeptide amino acid sequence of Fig. 45.
 - 39. The polypeptide of claim 38 wherein the light chain amino acid sequence comprises amino acids 1-219 of the light chain amino acid sequence of Fig. 45.

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- 40. The polypeptide of claim 38 that further comprises a heavy chain amino acid sequence comprising amino acids 1-230 of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B.
- 41. The polypeptide of claim 40, wherein the heavy chain amino acid sequence is fused at its C-terminus to a leucine zipper amino acid sequence.
 - 42. The polypeptide of claim 41, wherein the leucine zipper sequence comprises amino acids 231-275 of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B.
- 35 43. The polypeptide of claim 35 that is an antibody fragment selected from the group consisting of Fab, Fab', Fab'-SH, Fv, scFv and F(ab') 2.

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44. The polypeptide of claim 38 that is a F(ab') 2 antibody fragment, wherein the antibody fragment comprises a first heavy chain amino acid sequence and a second heavy chain amino acid sequence each comprising amino acids 1-238 of the heavy chain polypeptide amino acid sequence of Figs. 37A-37B, and wherein each of the Cys residues at positions 231 and 234 in the first heavy chain amino acid sequence is in a disulfide linkage with the identical Cys residue in the second heavy chain amino acid sequence.

45. The polypeptide of claim 38 that is a Fab' or Fab'-SH antibody fragment, wherein the antibody fragment comprises a heavy chain amino acid sequence comprising amino acids 1-233 of the heavy chain polypeptide amino acid sequence of Fig. 53.

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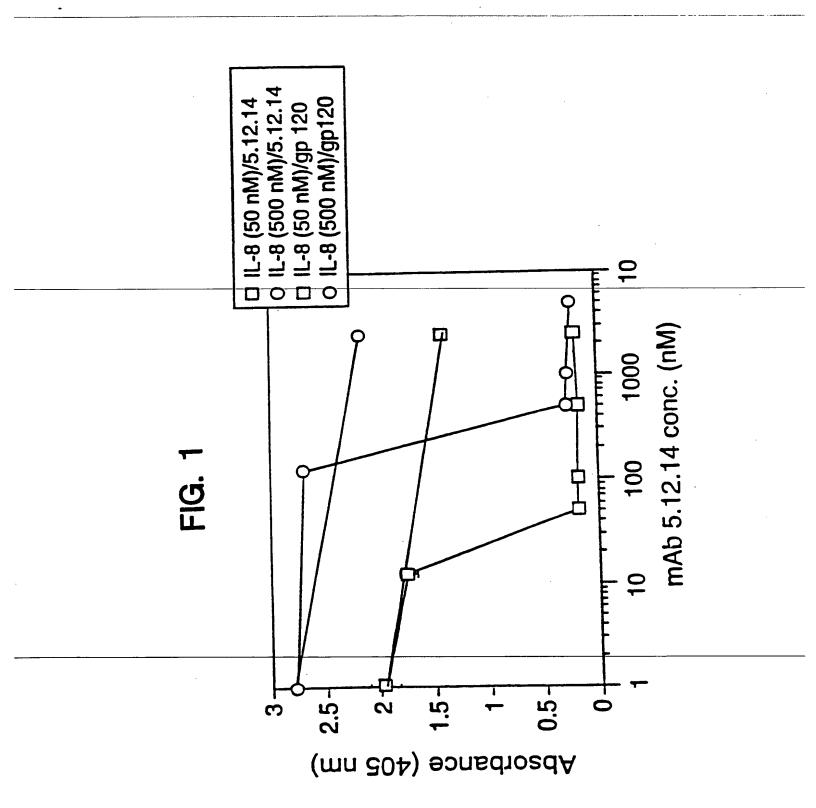
- 46. The polypeptide of claim 35 that is an antibody.
- 47. A nucleic acid molecule that comprises a nucleic acid sequence encoding the polypeptide of claim 35.

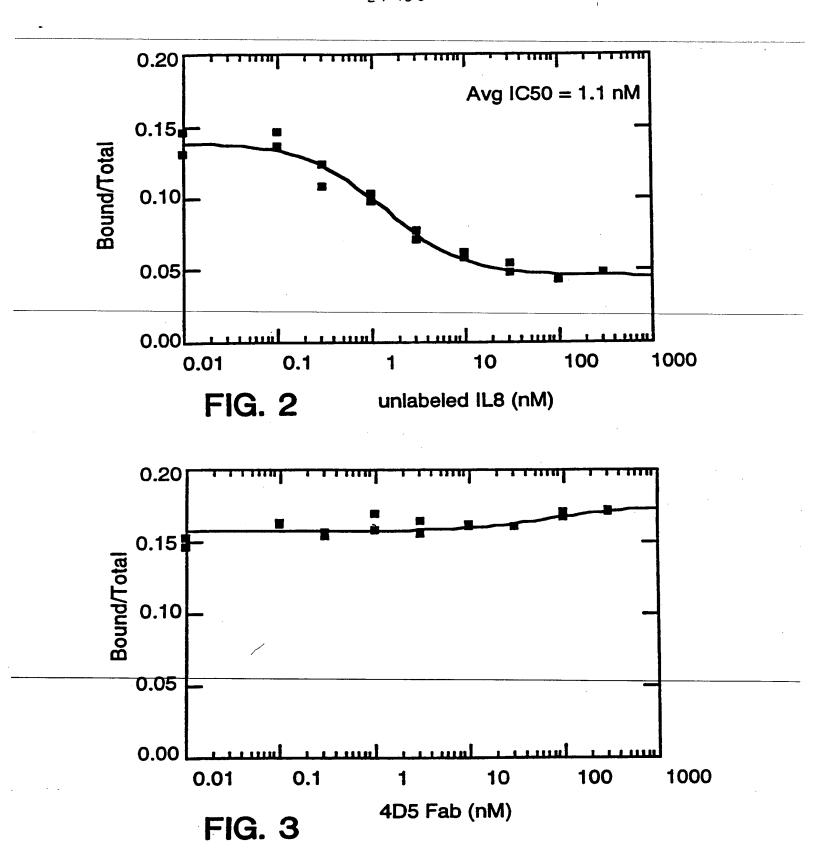
15

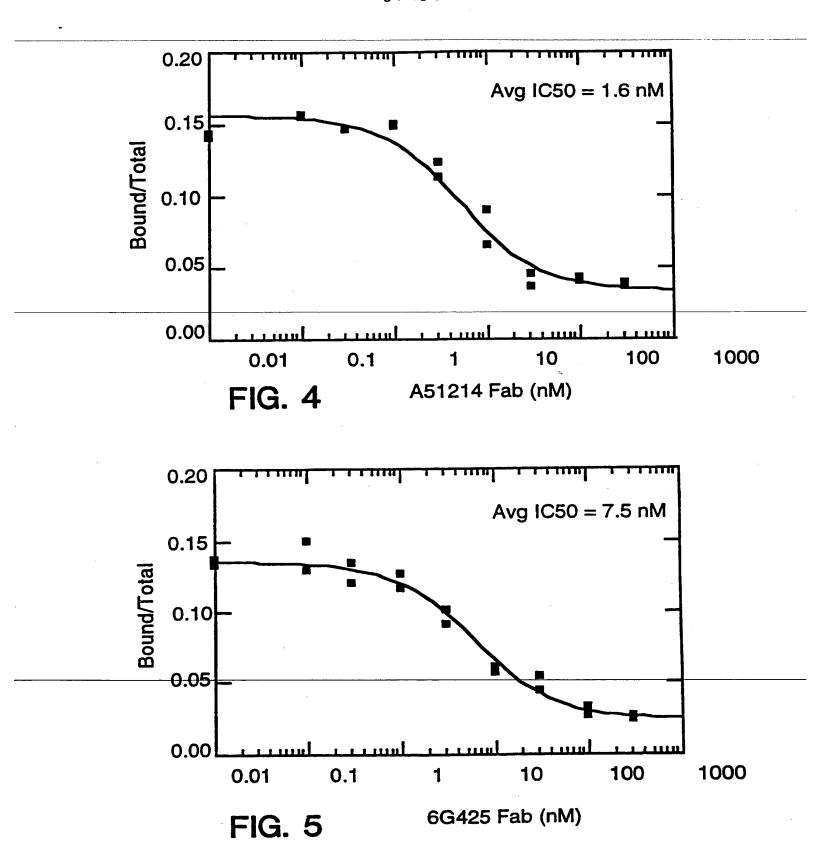
- 48. An expression vector comprising the nucleic acid molecule of claim 47 operably linked to control sequences recognized by a host cell transfected with the vector.
 - 49. A host cell comprising the vector of claim 48.

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- 50. A method of producing a polypeptide, comprising culturing the host cell of claim 49 under conditions wherein the nucleic acid sequence is expressed, thereby producing the polypeptide, and recovering the polypeptide from the host cell.
- 51. A composition comprising the polypeptide of claim 35 and a carrier.
 - 52. The composition of claim 51 that is sterile.







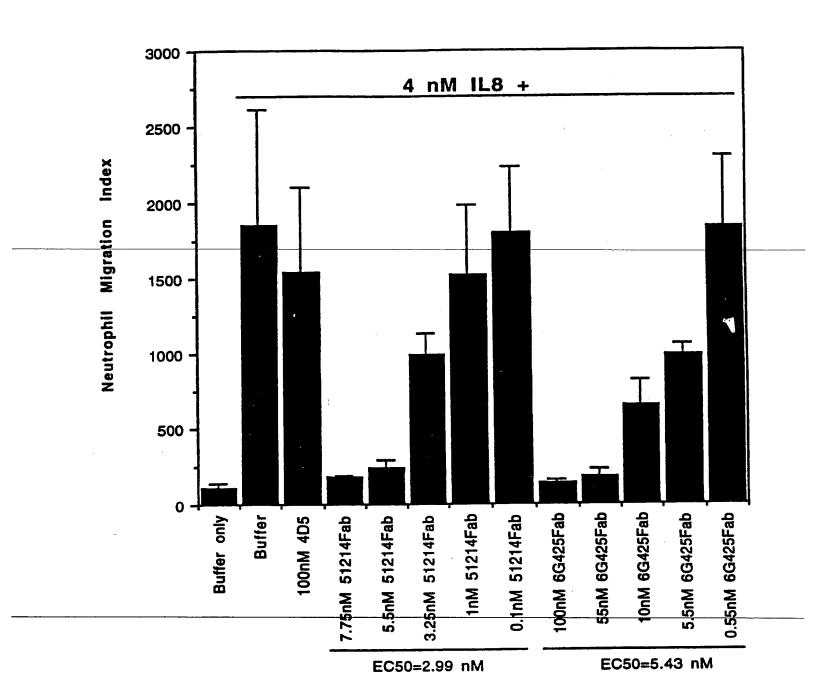


FIG. 6

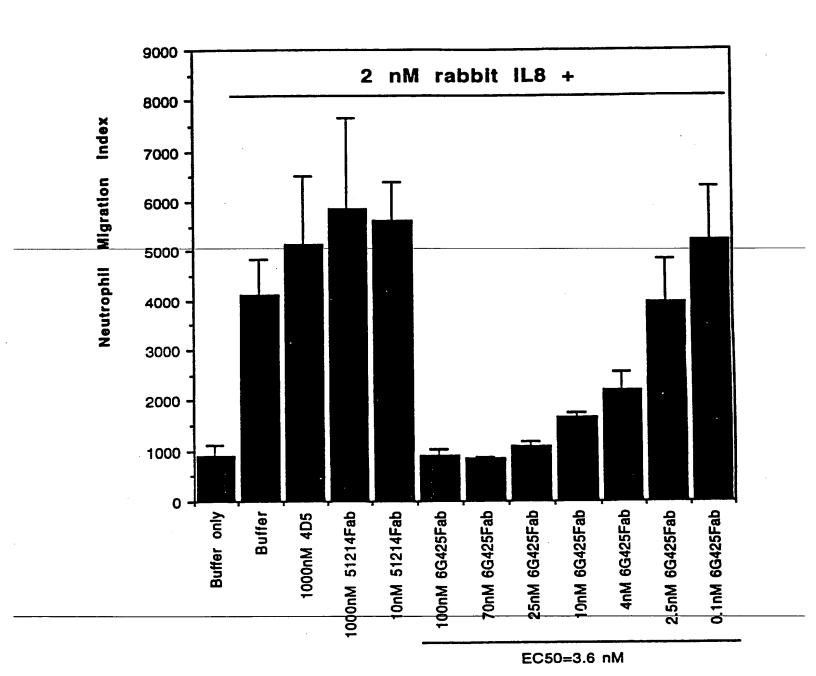
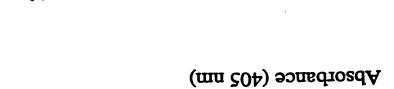
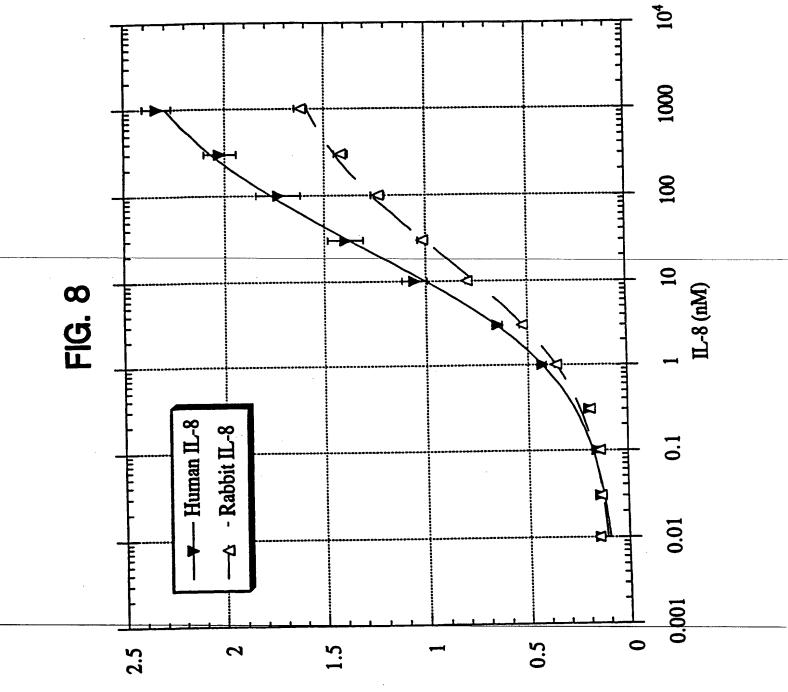
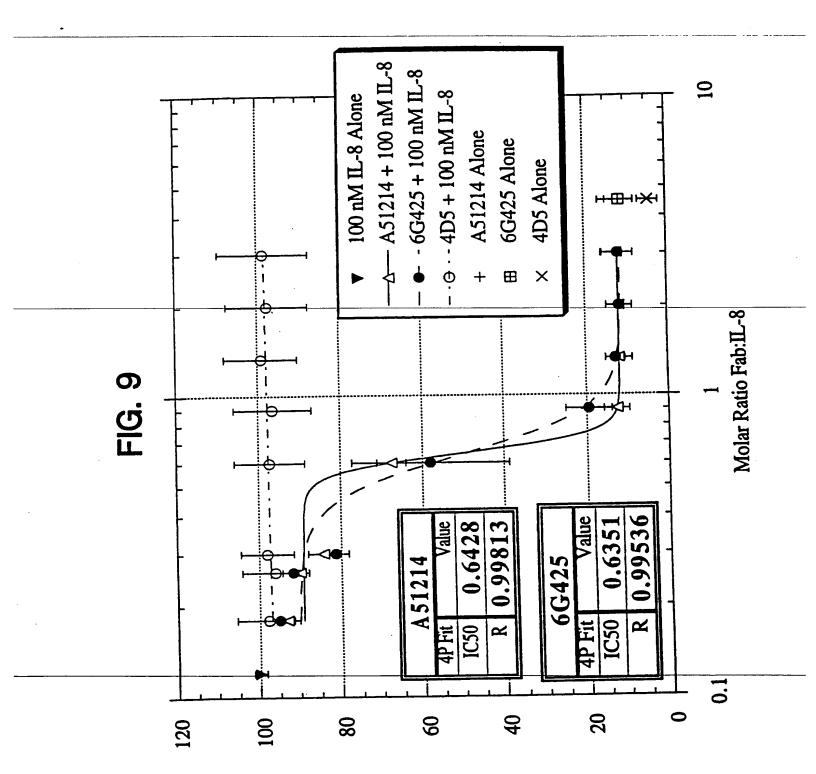


FIG. 7

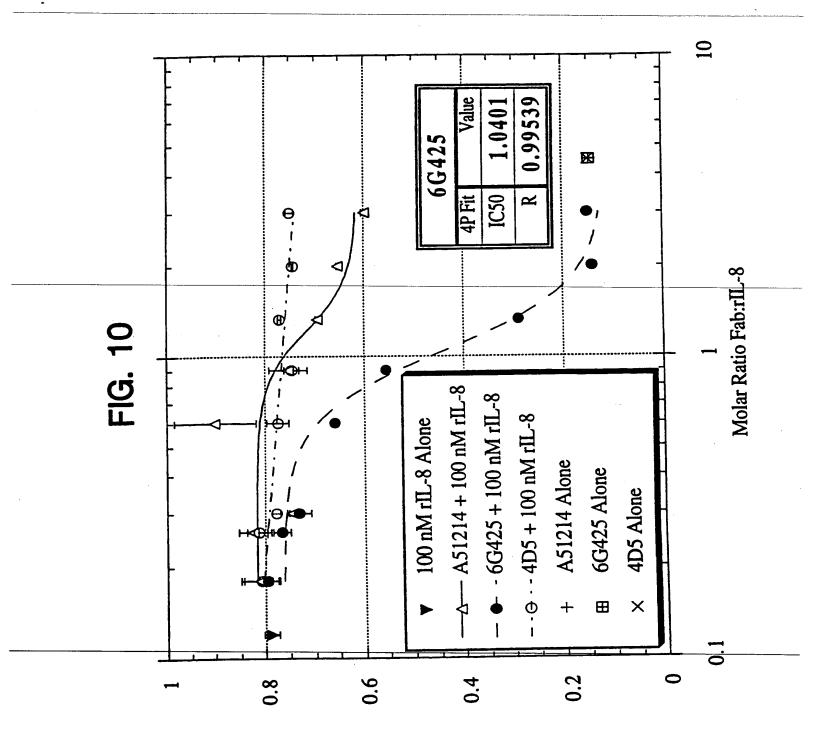


SUBSTITUTE SHEET (RULE 26)

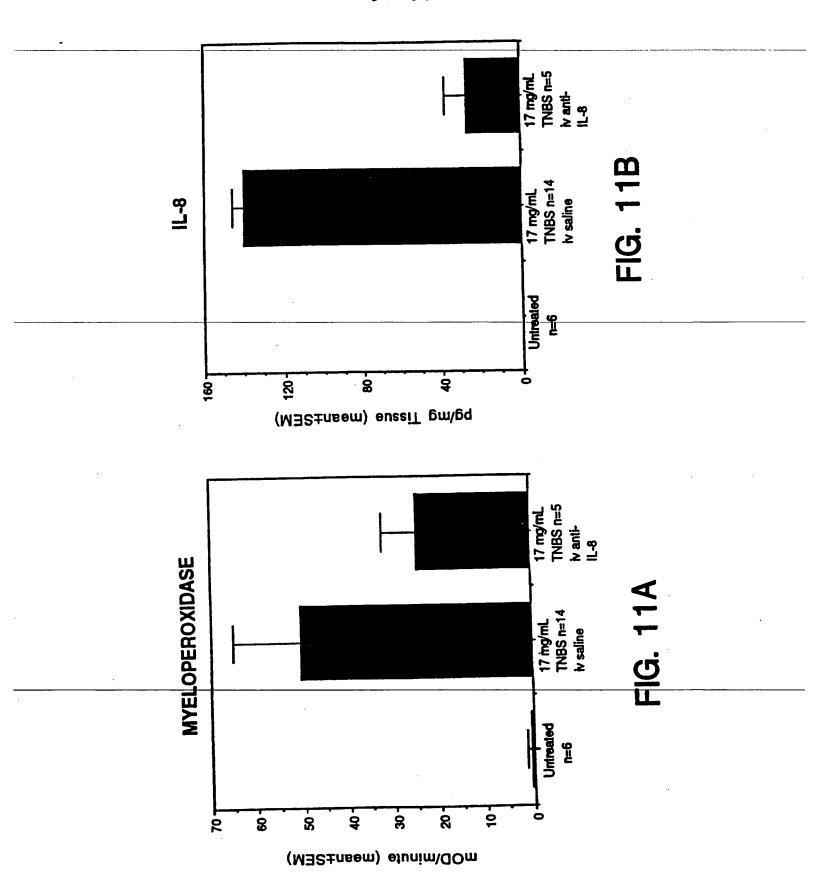




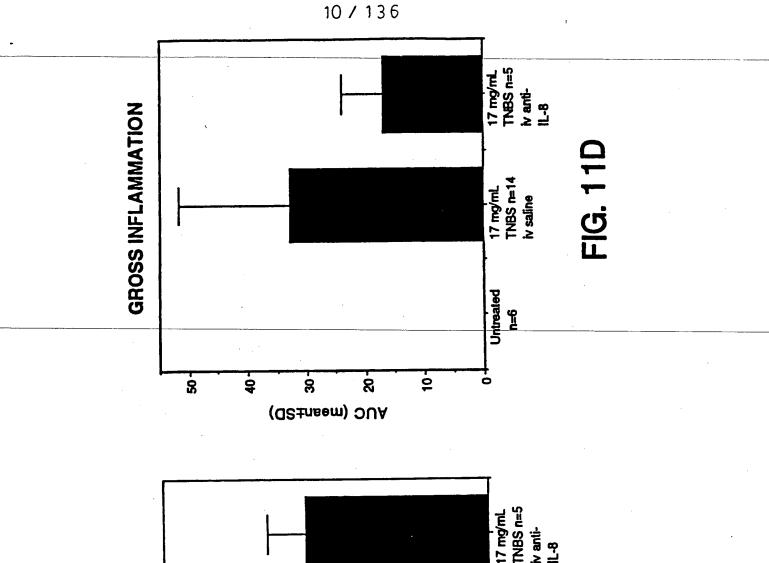
% IL-8-Stimulated Elastase Release

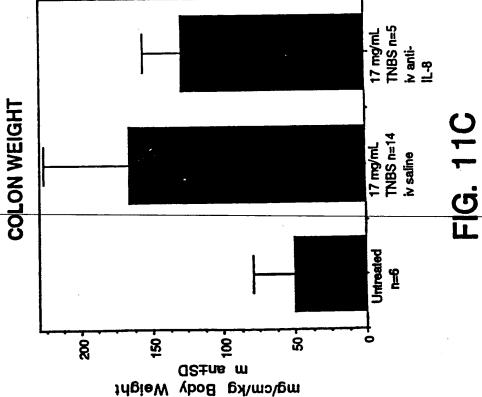


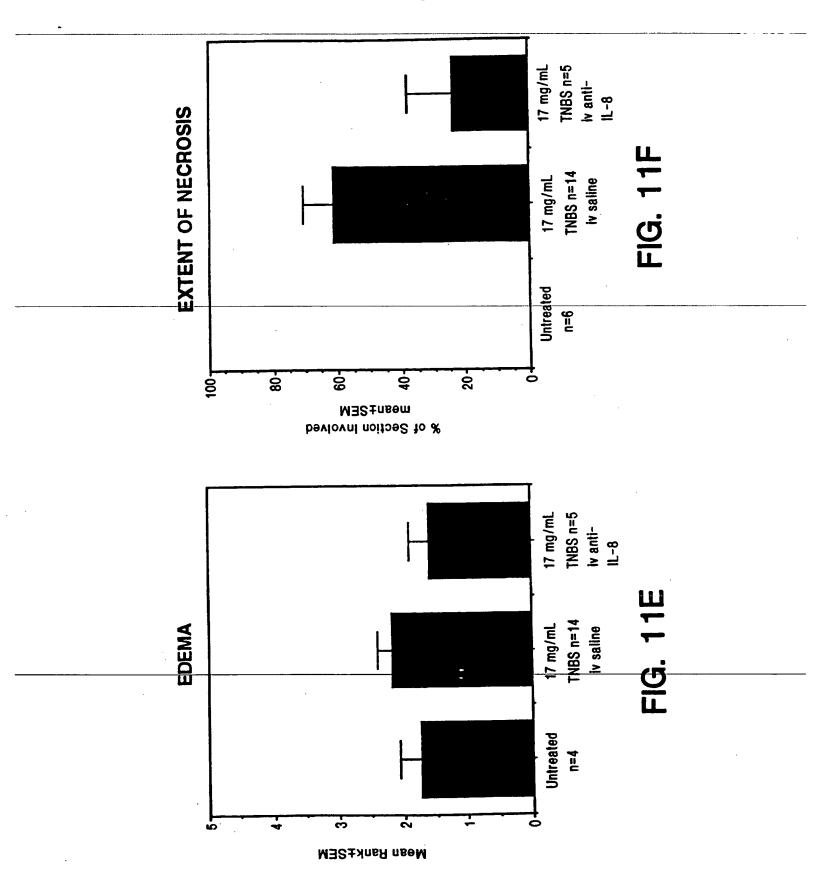
Absorbance (405 nm)

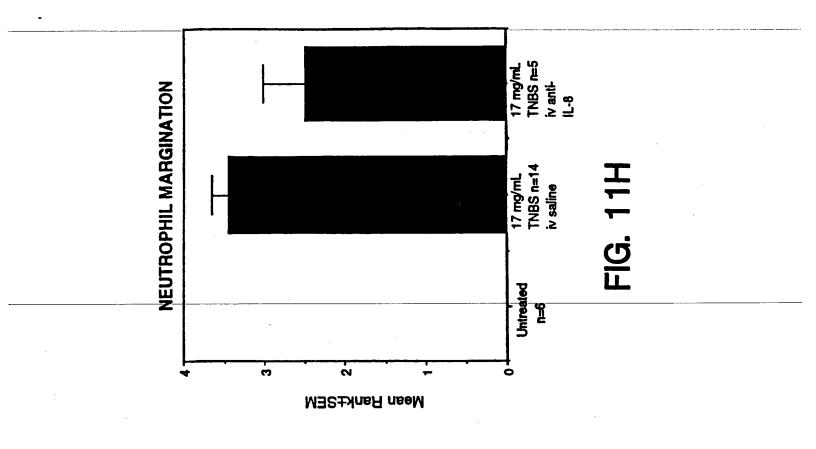


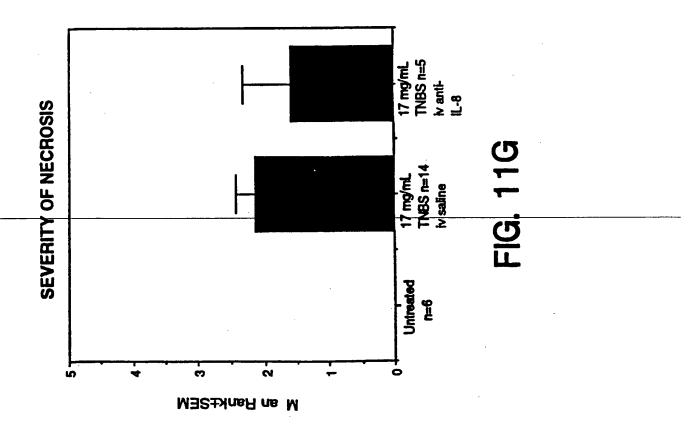


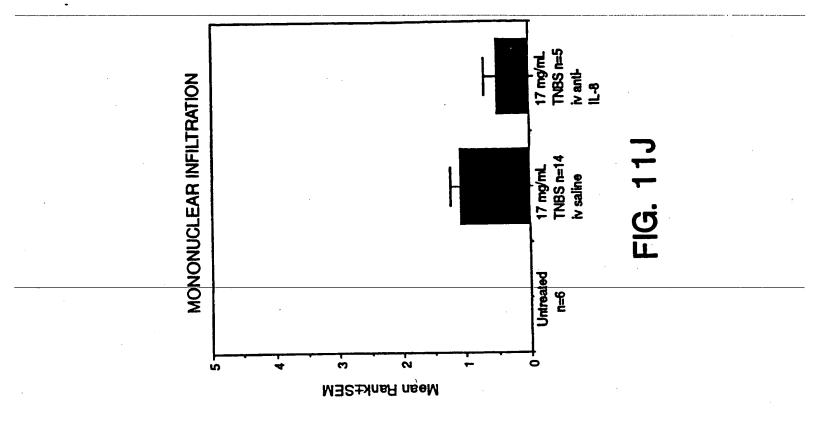


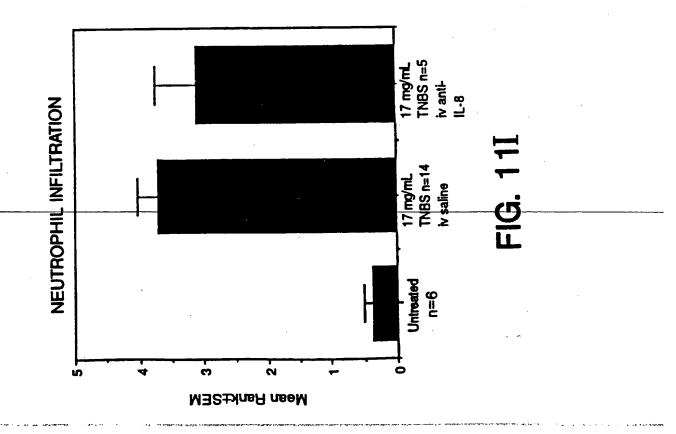


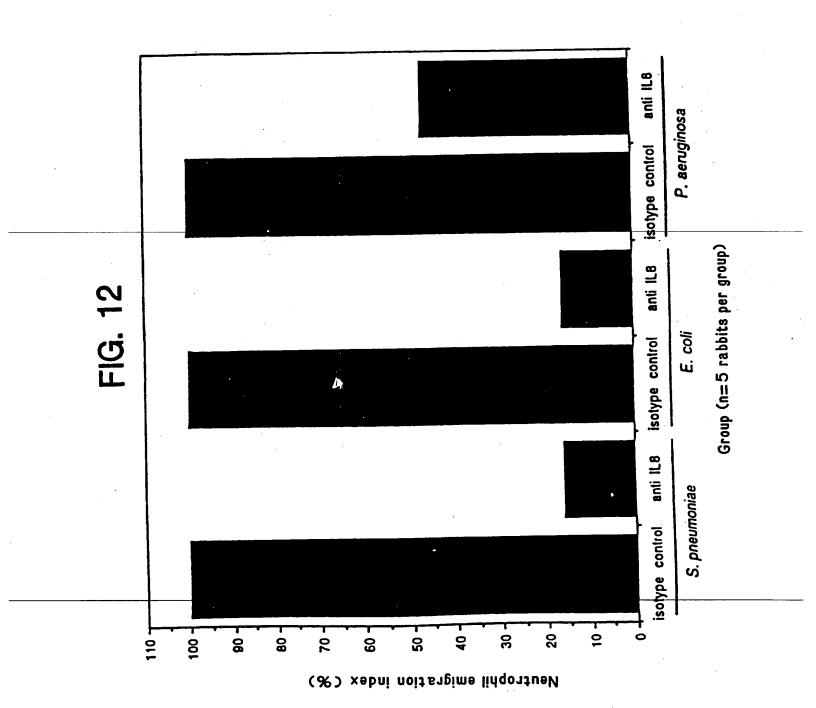












Light Ch	nain Primers:		
MKLC-1,	22mer	FIG. 13	
5 '	CAGTCCAACTGTTC	AGGACGCC 3'	
MKLC-2,	22mer		
5 '	GTGCTGCTCATGCT	GTAGGTGC 3'	
MKLC-3,	23mer		
5 '	GAAGTTGATGTCTT	GTGAGTGGC	3'
_	hain Primers: 1, 24mer		
5 '	GCATCCTAGAGTCA	.CCGAGGAGCC	3 '
IGG2AC-	2, 22mer		
5'	CACTGGCTCAGGGA	AATAACCC 3	
IGG2AC-	3, 22mer		
5 '	GGAGAGCTGGGAAG	GTGTGCAC 3	•

FIG. 14

Light chain forward primer

SL001A-2 35 mer

5' ACAAACGCGTACGCT GACATCGTCATGACCCAGTC 3' T T T

T T

Light chain reverse primer

SL001B 37 mer

5' GCTCTTCGAATG GTGGGAAGATGGATACAGTTGGTGC 3'

Heavy chain forward primer FIG. 15

SL002B 39 mer

5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTTTTGGC 3'

T C
G
A

Heavy chain reverse primer

SL002B 39-MER

5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTTTTTGGC 3'

T
A
G

CAGGGTCAGC GTCCCAGTCG R V S	ACAGAAACCA TGTCTTTGGT Q K P	GTCCCTGAT CAGGGACTA V P D	TGTGCAGTCT ACACGTCAGA V Q S	GTTCGGTCCT CAAGCCAGGA F G P	CATCTTCCCA GTAGAAGGGT I F P	
		GATTTACTCG TCATCCTACC GGTACAGTGG AGTCCCTGAT CTAAATGAGC AGTAGGATGG CCATGTCACC TCAGGGACTA I Y S S Y R Y S G V P D CDR #2	CCATCAGCCA TG GGTAGTCGGT AC I S H V		GGGACCAAGC TGGAGTTGAA ACGGGCTGAT GCTGCACCAC CAACTGTATC CATCTTCCCA CCCTGGTTCG ACCTCAACTT TGCCCGACTA CGACGTGGTG GTTGACATAG GTAGAAGGGT G T K L E L K R A D A A P P T V S I F P	FIG. 16
GACATTGTCA TGACACAGTC TCAAAATTC ATGTCCACAT CAGTAGGAGA CTGTAACAGT ACTGTGTCAG AGTTTTTAAG TACAGGTGTA GTCATCCTCT D I V M T Q S Q K F M S T S V G D	GTCACCTGCA AGGCCAGTCA GAATGTGGGT ACTAATGTAG CCTGGTATCA CAGTGGACGT TCCGGTCAGT CTTACACCCA TGATTACATC GGACCATAGT V T C K A S O N V G T N V A W Y Q CDR #1	TAAATGAGC AGTAGGATGG CITAAATGAGC AGTAGGATGG CITAAATGAGC AGTAGGATGG CITAAATGAGATGG CITAAATGAGATGG CITAAATGAGATGG CITAAATGAGATGG CITAAATGAGATGG CITAAAATGAGATGG CITAAAATGAGATGG CITAAAATGAGATGG CITAAAATGAGATGG CITAAAATGAGATGG CITAAAAATGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAA	TTCACTCTCA AAGTGAGAGT F T L T	CTGTCAGCAA TATAACATCT ATCCTCTCAC GACAGTCGTT ATATTGTAGA TAGGAGAGTG C Q Q Y N I Y P L T	ACGGGCTGAT GCTGCACCAC CAACTGTATC TGCCCGACTA CGACGTGGTG GTTGACATAG R A P P T V S	<u>u</u>
TCAAAAATTC AGTTTTTAAG Q K F	GAATGTGGGT CTTACACCCA N V G * * * CDR #1	GATTTACTCG CTAAATGAGC I Y S			ACGGGCTGAT TGCCCGACTA R A D	
GACATTGTCA TGACACAGTC CTGTAACAGT ACTGTGTCAG D I V M T Q S	CA AGGCCAGTCA GT TCCGGTCAGT K A S O * * * * *	CTAAAGCACT GATTTCGTGA K A L	GCAGTGGATC CGTCACCTAG S G S	GAAGACTIGG CAGACTATTT CTTCTGAACC GTCTGATAAA E D L A D Y F	GGGACCAAGC TGGAGTTGAA CCCTGGTTCG ACCTCAACTT G T K L E L K	
GACATTGTCA CTGTAACAGT D I V M	GTCACCTGCA CAGTGGACGT V T C K	GGGCAATCTC CCCGTTAGAG G Q S P	CGCTTCACAG GCGAAGTGTC R F T G			BstBI CCATTCGAA GGTAAGCTT P F E
ਜ ਜ	61 21	121	181 61	241	301	361

	1													GGTG							
	1	AAG	SATA	ACGA	TG	TTT	GCG	CA	TGCG	ACT E	V V	CGI Q		CCAC V	CT E	CAG S	ACC(CC G	G	GAA L	TCA V
	61													CTCT GAGA							
	4.3																			ATC	AAT
	13	P	P	G	G	S	L	K	L	S	С	A	A	S	<u>G</u>	F		F	s	<u> </u>	<u> </u>
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	181	TAA	ТАА	TGGT	GA'	TAG	CAC	СT	ATTA	TCC	AGA	CAG	тст	GAAG	GG	CCG	እ ጥ ፕ(2A	CCAT	СТС	CCG
														CTTC							
	53		N	_	D	S	T	Y	Y	P	D	S	V	ĸ	G	R	F	T	I	S	R
		*	*	*	*	*	*	*	*	*	*	*	*	*							
						(CDR	#2	2												
:	241	AGA	CAA	TGCC	AA	GAA	CAC	CC	TGTA	CCT	GCA	AAT	GAG	CAGT	CT	GAA	GTC!	ľG	AGGA	CAC	AGC
		TCT	GTT.	ACGG	TT	CTTC	GTG	GG	ACAT	GGA	CGT	TTA	CTC	GTCA	GA	CTT	CAG	AC	TCCT	GTG	TCG
	73	D	N	A	K	N	T	L	Y	L	Q	M	S	S	L	K	S	E	D	T	A
:	301	CAT	GTT	TTAC	TG:	rgci	AAG	A G	CCCT	CAT	TAG	TTC	GGC'	TACT	TG	GTT.	rgg:	ГT	ACTG	GGG	CCA
		GTA	CAA	AATG	AC	ACG:	rtc:	rc	GGGA	GTA	ATC	AAG	CCG.	ATGA	AC	CAA	ACC	AA	TGAC	CCC	GGT
	93	M	F	Y	C	A	R	A	L_	I	S	S_	_A_	T	W	F	G	Y	W	G	Q
								*	*	*	*	*	*	*	*	*	*	*			
											CI	OR#	3								
	361	ACC	GAC	יריתכ	GTO	רם בי	רכתי	-Th	СТСС	ACC		ממ	אאר	AGCC	CC	איירי	אניבאו	- ф			
•														TCGG							
•	113	G	T.	L	v	T	V	S	A	A	K	T	T	A	P	S	V	Y			
			-					_										-		· ·	

FIG. 17

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ApaI

ATCC GGG

TAGGCCC

FIG. 18

VL.front	31-MER		
5' ACAA <u>ACGCGT</u> VL.rear 31-M	ACGCI <u>GATATC</u> GTCATCHIC	3'	
5' GCAGCATCAC	SCTC <u>TTCGAA</u> GCTCCAGCTTGG	3'	
VH.front.SPE	21-MER		
5' CCACTAGTAG	CGCAAGTTCACG	3 '	
VH.rear 33-M	ER		,
5 ' GATGGGCCC	rtggtggaggctgcagagacag1	.'G	3

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								21	/ 1	136											
1	ATG	AA(GAA	GA	ATAT	CGC	TT	TČT'	TCT'	rgca	TCT	PTAT	TTC	G	TTTTI	TCT	TA	TGC'		AAAC	
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101	GTC	TT.	rgg	TC	CCGT	TAG	AGG	ATT	TCG'	TGAC	TA	YTA	GAGO	CA	GTAG	GAT	GC	CAT	GTC.	ACCI	ľ
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	TTC	AA	GAT	AG	GGTC	TCT	CCG	GTI	TCA	TGTC	AC	CTT	CCA	CC	TATT	GCG	GGA	GG"	PATT	CCC.	A
138	N	F	Y	P	R	E	A	K	V	Q	W	K	V	D	N	A	L	Q	S	G	
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541	AAC	TC	CCA	GG	AGAG	TGT	CMC	AGA	IGCA ICCT	CONC	AG	CMM	CCT	CA CT	GCAC	TAD!	いかい	GG!	CAC LCAC	てない	G
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	1	ΑТ	GAA	AAA	GA	ATATCGCATT			TCTTCTTGCA			TCTATGTTCG				$\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}\mathbf{T}$	TCI	TA!	TGCTACAAAC				
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	61	GC	GTA	CGC	TG	AGGT	GCA	GCT	GGT	SGA (STCT	GGC	GGA	<i>i</i> GGC	T	TAGTO	SCCC	SCC.	TGG	<i>YCCC</i>	TCC		
		CG	CAT	GCG	AC	TCCA	CGT	ĊGA	CCA	CCT	CAGA	CCC	CCI	rccc	JA .	ATCAC	CGGC	CGG	ACC:	rccc	:AGG		
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		GA	CTT	TGA	GA	GGAC.	ACG'	rcg	GAG	ACC.	PAA 1	TAT	'AAC	STC	\T	CAATA	<i>y</i> CC6	TA	CAG	AACC	CAA		
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	101	CG	CCA	GAC	TC	CAGG	CAA	GAG	ССТССАСТТС			GTCGCAACCA			CA	TTAATAATAA			TGGTGATAGC				
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		TG	GAT.	AAT	AG	GTCT		ACA								GGGC:							
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		TG	GGA	CAT	GG	ACGT	TTA	CTC	GTC	AGA	CTTC					GTCG	GTA	CAA					
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	421	GT	СТС	TGC	AG	CCTC	CAC	CAA	GGG	CCC.	ATCG	GT	CTT(CCC	CC	TGGC.	ACC	CTC	CTC	CAA	GAGC		
		CA	CVC	ACC.	mC.	GGAG	CTG	ىلىملى	CCC	GGG	TAGC	CAC	GAA	GGG	GG	ACCG	TGG	GAG	GAG	GTT	CTCG		
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	481	AC	CTC	TGG	GG	GCAC	AGC	GGC	CCT	GGG	CTGC	CT	GGT(CAA	GG	ACTA	CTT	CCC	CGA	ACC	GGTG		
		тс	GAG	ACC	CC	CGTG	TCG	CCG	GGA	CCC	GACG	GA	CCA	GTT	CC	TGAT	GAA	GGG	GCT	TGG	CCAC		
	138						Δ	Δ	T.	G		Τ.	V	K	D	Y	F	P	E	P	V		
	120	7	3	G	G	-	71	•		•	_		•	••	_	_	_	-					
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	541	AC	GGT	GTC	GT	GGAA	CTC	AGG	CGC	CCT	GACC	AG	CGG	CGT	GC	ACAC	CTT	CCC	GGC	.161	CCIM		
*		TG	CCA	CAG	CA	CCTT	GAG	TCC	GCG	GGA	CTGG	TC	GCC	GCA	.CG	TGTG	GAA	GGG	CCG	ACA	GGAT	•	
	158						S	$\boldsymbol{G}$	A	L	$m{T}$	S	G	V	H	T			A				
	100	4	•	_	••		_	_	••	_	_	_	_	•									
	<b>.</b>						~~*			~ T	~~~	~~	~~~	~~~		mece		~~~	CAC	تصسح	CCCC		
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		GT	CAG	GAG	TC	CTGA	GAT	GAG	GGA	GTC	GTCG	CA	CCA	CTG	GC	ACGG	GAG	GTC	GTC	AA.	CCCG	,	
	178	0	S	S	$\boldsymbol{G}$	L	Y	S	L	S	S	V	$\boldsymbol{v}$	T	$\boldsymbol{v}$	P	S	S	S	L	G		
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- 661 ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGCA ACACCAAGGT GGACAAGAAA
  TGGGTCTGGA TGTAGACGTT GCACTTAGTG TTCGGGTCGT TGTGGTTCCA CCTGTTCTTT
  198 T O T Y I C N V N H K P S N T K V D K K
- 721 GTTGAGCCCA AATCTTGTGA CAAAACTCAC ACATGA CAACTCGGGT TTAGAACACT GTTTTGAGTG TGTACT 218 V E P K S C D K T H T O

FIG. 20B

Hight Ci	nain Filmers.	
MKLC-1,	22mer	
5 '	CAGTCCAACTGTTCAGGACGCC 3'	
MKLC-2,	22mer	
5 '	GTGCTGCTCATGCTGTAGGTGC 3'	
MKLC-3,	23mer	
5'	GAAGTTGATGTCTTGTGAGTGGC	3'
Heavy Cl	hain Primers:	
IGG2AC-	1, 24mer	
5 '	GCATCCTAGAGTCACCGAGGAGCC	3 '
IGG2AC-	2, 22mer	
5 '	CACTGGCTCAGGGAAATAACCC 3'	
IGG2AC-	3, 22mer	
5'	GGAGAGCTGGGAAGGTGTGCAC 3'	

FIG. 21

Light chain forward primer

6G4.light.Nsi 36-MER

5' CCAATGCATACGCT GAC ATC GTG ATG ACC CAG ACC CC 3'

T T T T A A

Light chain reverse primer

6G4.light.Mun 35-MER

5' AGA TGT CAA TTG CTC ACT GGA TGG TGG GAA GAT GG 3'

FIG. 22

### Heavy chain forward primer

6G4.heavy.Mlu 32-MER

5' CAAACGCGTACGCT GAG ATC CAG CTG CAG CAG 3'
T C

Heavy chain reverse primer

SL002B 39-MER

5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC 3

Τ.

A

G

# FIG. 23

BNBDOOID- 2000 000700000 1 5

70 G ATATCGTGAT GACACAGACA CCACTCTCCC TGCCTGTCAG TCTTGGAGAT C TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA D I V M T Q T P L S L P V S L G D 121 CAGGCCTCCA TCTCTTGCAG ATCTAGTCAG AGCCTTGTAC ACGGTATTGG AAACACCTAT GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGGAACATG TGCCATAACC TTTGTGGATA 18 Q A S I S C R S S O S L V H G I G * * CDR #1 181 TTACATTGGT ACCTGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC AATGTAACCA TGGACGTCTT CGGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTTCAAAGG 38 L H W Y L Q K P G Q S P K L L I Y **CDR #2** 241 AACCGATTTT CTGGGGTCCC AGACAGGTTC AGTGGCAGTG GATCAGGGAC AGATTTCACA TTGGCTAAAA GACCCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT 58 N R F S G V P D R F S G S G T D F T 301 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAGTACA GAGTCCTAGT CGTCTCACCT CCGACTCCTA GACCCTGAAA TAAAGACGAG AGTTTCATGT 78 L R I S' R' V E A E D L G L Y F C S CDR #3 361 CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC TGAAACGGGC TGATGCTGCA GTACAAGGCG AGTGCAAGCC ACGACCCTGG TTCGACCTCG ACTTTGCCCG ACTACGACGT 98 H V P L T F G A G T K L E L K R A D A A 421 CCAACTGTAT CCATCTTCCC ACCATCCAGT GAGCAATTGA GGTTGACATA GGTAGAAGGG TGGTAGGTCA CTCGTTAACT 118 P T V S I F P P S S E Q L K

135 I

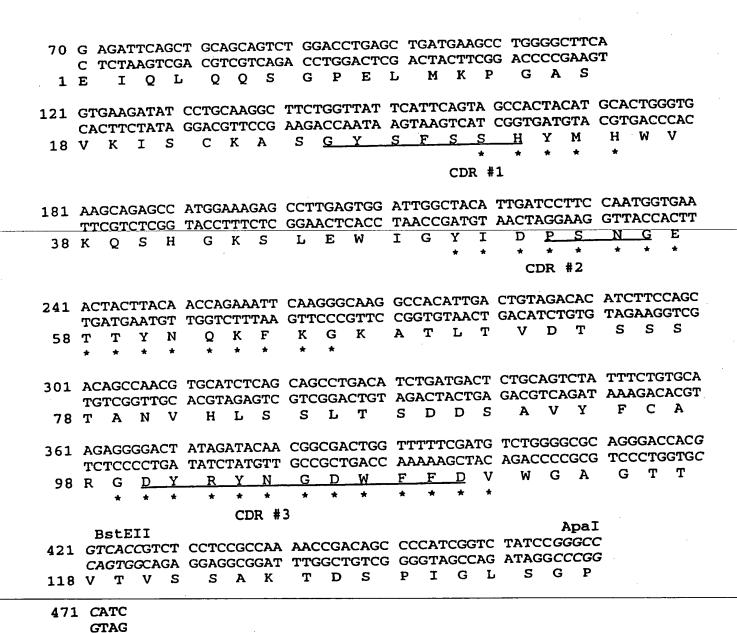


FIG. 25

5' CTTGGTGGAGGCGGAGACG 3'

Mutagenesis Primer for 6G425VL

DS/VF 38MER

5' GAAACGGGCTGTTGCTGCACCAACTGTATTCATCTTCC 3'

SYN.BstEII 31 MER

5' GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 3'

SYN.Apa 22 MER

5' CTTGGTGGAGGCGAGGAGACG 3'

FIG. 26

									m	naa.	mem	13 mc	mmc	· ·	ուրուու	ጥርጥ	י יחמ	ייברית	מימי	ייעגג	
1_	ATY	GAA	GAA	GA	ATATO	CCC	7.1.1.	TCT.	CT	NCCT	AGA	TATE	סממי	.G :	TTTTT AAAAA	AGA	TA	ACGA	TGT TGT	ጥጥ ጉጥል	
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-23	M	K	K	14	1	A	F		u		_	••	•	•	-	_	_		_		
<b>6</b> 1	CC	מידים	CGC	TC	ארארע	ገርጥር	TAF	GAC	ACAG	GACA	CCA	CTC	TCC	C:C	TGCCI	GTC	'AG	TCTI	rgga	GAT	
91	CC	ጥልጥ የ	പ്പുട്ട വേധ	AC.	TATA	CAC	TA	CTG'	rgr	CTGT	GGT	GAC	AGG	G	ACGG?	CAG	TC	<b>AGA</b>	ACCI	CTA	
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121	CA	GGC	CTC	CA	TCTC	TTGC	CAG	ATC'	rag'	<b>TCAG</b>	AGC	CTI	CTA	VC	ACGGT	r <b>ta</b> r	'GG	AAA	CACC	TAT	
	GT	CCG	GAG	GT	AGAG	AACC	STC	TAG	ATC	AGTC	TCG	GAZ	CAI	ď	TGCC	AATA	rcc	TTTC	STGG	ATA	
18	Q	A	S	I	S	C	R	S	S	0	S	<u>L</u>	<u></u>	H	G_	<u> </u>	G_	_N_	<u>T</u>	X	
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											C	CDR	#1								
181	TT	ACA	TTG	GT	ACCT	GCAC	AAE	GCC.	AGG	CCAG	TCI	rccz	AAAC	3C	TCCT	SATO	TA	CAA	AGTT	TCC	
															AGGA			GTT"	ICAA	LAGG	
38	L	H	W	Y	L	Q	K	P	G	Q	S	P	K	L	L	T	Y	K_	<u>-</u> Y	<u>.</u>	
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241	AA	CCG	ATT	TT	CTGG	GGT	CCC	AGA	CAG	GITC	AG	1.000	-AG1	10	GATC:	ちいい	אכ באת	ጥርጥ	7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	יייבארב.	
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78			I			v		A			L	G	L	Y	F	С	S	Q	S	<u>T</u>	
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																	CI	OR#	3		
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	GT	ACA	AGG	CG	AGTG	CAA									ACTT	TGC	CCG	ACA	ACG.	ACGT	
98	H_		P	_ <u>L</u>	T	F	G	A	G.	T	K	L	E	L	K	R	A	V	A	A	
	*	*	*	*	*																
421	CC	'AAC	TGT	TAT	TCAT	CTT	CCC	ACC	ATC	CAGT	GA	GCA	ATT	GA	AATC	TGG	AAC	TGC	CTC	1G11.	
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241		2001		בנה ענה	GCCC	ידיים	GAG	GGT	rcci	CTCA	CA	GTC	TCT	'CG	TCCI	CTC	GTT	CC	rgtc	:GTG	;
159	A	JUGI T,	 0	S	G	N	S	0	E	S	V	$oldsymbol{T}$	E	Q	D	S	K	D	S	T	
601	T	ACAC	GCC?	rca	GCAG	CAC	CCT	GAG	GC.	rgago	: AA	AGC	AGA	CT	ACG	\GA#	LACA	CA	AAGI	CTAC	
	יע	ቦር፣ጥር	CG	AGT	CGTC	GTG	GGA	CTO	3CG2	ACTCG	TI	TCC	STCI	GA	TGC	rcti	rtgi	'GT"	rtc:	GATO	3
178	Y	S	L	S	S	$oldsymbol{T}$	L	\boldsymbol{T}	L	S	K	A	D	Y	E	K	H	K	V	Y	
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CAACAGGGGA GTIGICCCCI N R G	
CAAAGAGCITI GTTTCTCGAA KSF	
TCGCCCGTCA AGCGGGCAGT S P V T	FIG 27B
GGGCCTGAGC CCCGGACTCG G L S	E E
661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGAA CGGAGGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT 198 A C E V T H Q G L S S P V T K S F N R G	
CGAAG GCTTC E V	TTAA AATT O
GCCTG CGGAC A C	721 GAGTGTTAA CTCACAATT 218 E C O
198	721

														_		~~~	13 M	maar	1202		
1	TA	GAA	AAA	GA-	ATAT	CGC	ATT_	TCT	TCT	TGCA_	TC	PATC	TIC	G	J.T.T.T.T.		AT.	ACCI	MC		-
	_				TATA						AG	ATAC	:AAG	C	AAAAA	AGP C				'N	
-23					-	A	_	_	L						F		I	A	-		
61	GC	GTA	CGC	TG	AGAT TCTA	TCA(GCT	GCA	GCA CCT	GTCT	GGZ	ACCI rgg <i>i</i>	'GAG 'CTC	C G	TGAT(ACTA(SAAC OTTC	SCC CGG	TGG(GC! CCG!	rtca Aagt	
•		Y				0			Q		G	P	E	L	M	K	P		A		
_						_		_	-								- m	001	~m~		
121	GT	GAA CTT	GAT. CTA	AT TA	CCTG	CAA(GTT(GC CCG	TTC	TGG ACC	TTAT AATA	TC/)TTA OAA1	:AGT STCA	A T	CGGT)ATC YTAE	:AT	CGT	SAC(CCAC	
1.8		ĸ				ĸ				Υ						Y	M	H	W		
	•	••	_	-										*	*	*	*	*			
													CDR	#	1						
101	7 7	CCN	GAG	CC	ATGG	AAA	DAS	ССТ	тса	GTGG	AT'	rgg	TAC	:A	TTGA:	rcc:	rtc	CAA	ľGG'	TGAA	
TOT	ינועף באנז	CCT CCT	CTC	GG	TACC	TTT(CTC	GGA	ACT	CACC	TA	ACC	ATG	T	AACT	AGG/	AAG	GTT	ACC	ACTT	
38		Q	-			ĸ		L				G		I	D		S		G	E	
		×											*	*	*	*	*	*	*	*	_
															C	DR =	∦2				
241	AC	TAC ATG	TTA AAT	CA	ACCA TGGT	GAA!	TTA AA1	CAA	.GGG	CAAG	GC(CACI GTG:	OTTA OAA1	A T	CTGT	AGA(TCT(CAC GTG	ATC TAG	TTC AAG	CAGC GTCG	
58		T	Y	N	Q	K	F	K		ĸ					v		T	S		S	
	*	*	*	*	*	*	*	*	*												
301	AC	AGC	CAA	CG	TGCA	TCT	CAG	CAG	CCT	GACA	TC	TGA!	IGAC	T	CTGC	AGT	CTA	TTT	CTG	TGCA	
301	TG	TCG	GTT	GC	ACGT	AGA	GTC	GTC	GGA	CTGT	AG.	ACT	ACTO	A	GACG	TCA	GAT	AAA	GAC	ACGT	
78		A			Н				L			D		S	A	V	Y		С		
361	λG	ACC	CCA	СТ	ATAG.	АТА	CAA	CGG	CGA	CTGG	TT	TTT	CGAT	ľG	TCTG	GGG	CGC	AGG	GAC	CACG	
301	TC	TCC	CCT	GA	TATC	TAT	GTT	GCC	GCT	GACC	AA	AAA	GCT#	/C	AGAC	CCC	GCG	TCC	CTG	GTGC	
98	R		D	Y	R	Y	N			W		F		v		G		G		T	
,		*	*	*	*	*	*	*	*	*	*	*	*	*							
						CD	R #:	3													
421	GI	CAC	CGT	CT	CCTC	CGC	CTC	CAC	CAA	GGGC	CC	ATC	GGT	CT	TCCC	CCT	GGC	ACC	CTC	CTCC GAGG	
110	CA	GTG.	GCA T	AU.	S	GCG	omu C	GIG	27 I	CCCG	D	ZAG	CCA(JR F	AGGG	7.	A	P	S	S	
																				*	
481	AA	GAG	CAC	CT	CTGG	GGG	CAC	AGC	GGC	CCTG	GG	CTG	CCT	3G	TCAA	GGA	CTA TANK	CTI	CCC	CGAA	
					G																
541	CC	:GGT	GAC	:GG	TGTC	GTG	GAA	CTC	:AGG	CGCC	CT	GAC	CAG	CG	GCGI	'GCA	CAC	CTT	CCC	CGGCT	
																				CCGA	
158	P	V	T	V	S	W	N	S	G	A	L	T	S	G	V	H	T	F	P	A	
601																				CAGC	
	CA	LGGA	TGT	'CA	GGAG	TCC'	ľGA	GAT	GAG	GGAG	TC	GTC	GCA	CC	ACTO	GCA	'CGG	GAG	GT	CGTCG	
178	V	L	Q	· S	S	G	L	Y	S	L	S	S	V	V	T	V	P	S	S	S	
									FI	G.	2	8/	4								

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661 TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG GTTCCACCTG	721 AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GA TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CT $^{\prime}$ 218 K $^{\prime}$ $^$	
CTGCAACGT GACGTTGCA C N V	TTGTGACAA AACACTGTT C D K	Č
AGACCTACAT TCTGGATGTA T Y I	AGCCCAAATC TCGGGTTTAG P K S	
000 000 000	TTG AAC / E	
TTGGGCF AACCCGT	AAGAAAG TTCTTTG K K 1	
661	721	

Variable Light Chain Domain

	10 20 abcde 30 40
6G425	DIVMTQTPLSLPVSLGDQASISCRSSQSLVHGIGNTYLHWYLQKPGQSPKLLIY
m(t) 1	# # # # # # # # # # # # # # # # # # #
F(ab)-1	# ######
humĸI	DIQMTQSPSSLSASVGDRVTITCRASKTISKYLAWYQQKPGKAPKLLIY
	+++++++++
	L1
CG 435	50 60 70 80 90 100 YKVSNRFSGVPDRFSDSGSGTDFTLRISRVEAEDLGLYFCSQSTHVPLTFGAGTKLELKR
6G425	# # # ##### ### # # # # # # # # # # #
F(ab)-1	YKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTHVPLTFGQGTKVEIKR ## ###
humĸI	YSGSTLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQHNEYPLTFGQGTKVEIKR
Huma	
	++++++
	+++++
	L2

Variable Heavy Chain Domain

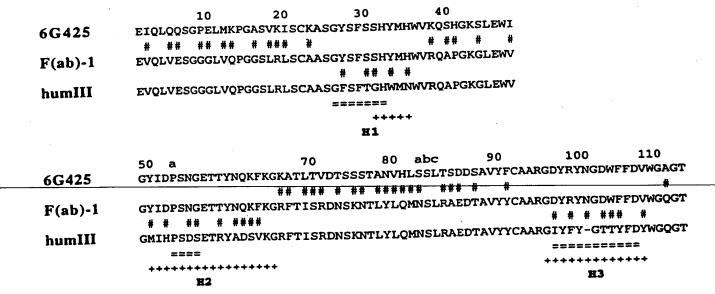


FIG. 29

PCT/US98/03337

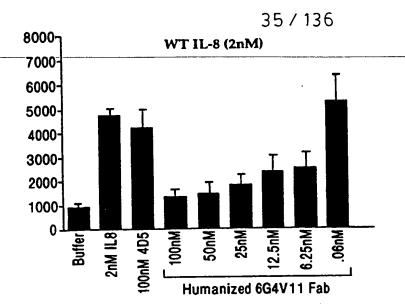


FIG. 30A

IC50~12nM

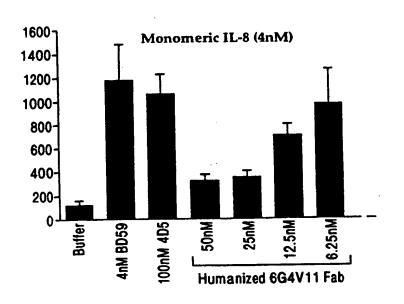
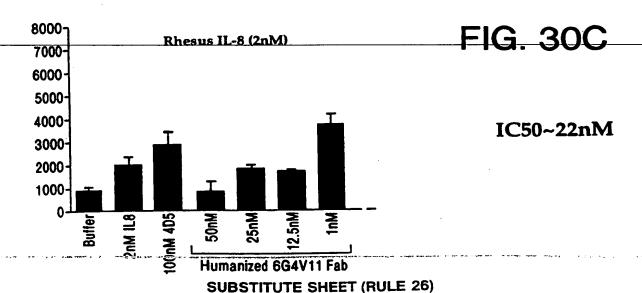


FIG. 30B

IC50~15nM



anti-IL-8 6G4.2.5V11 Light Chain Amino Acid Sequence of the humanized

ALOSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLT1SSLQPEDFATYYCSQST MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVT|ITCRSSQSLVHGIGNTY

anti-IL-8 6G4.2.5V11 Heavy Chain Amino Acid Sequence of the humanized

WVRQAPGKGL#WVGYIDPSNGETTYNQKFKGRFT**L**SRDNSKN†**A**YLQMNSLRAEDTAVYY PEPVTVSWNS¢ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSSJGTQTYICNVNHKPSNTK CARGDYRYNG DWFFD VWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF MKKNIAFLLA\$MFVFSIATNAYAEVQLVQSGGGLVQPGGSLRLSCAASGYSFSSHYMH VDKKVEPKSCDKTHT Amino Acid Sequence of the peptide linker and M13 Phage Coat (gene-III)

GLANGNGATGФFAGSSNSQMAQVGDGDNSPLMNNFRQYLPSL#QSVECRPFVFSAGKPY SGGGSGSGDF#YEKMANANKGAMTENADENALQSDAKGKLDSVATDYGAAIDGFIGDVS **EFSIDCDKINLFRGVFAFLLYVATFMYVFSTFANILRNKES**

FIG. 31A

										•	130											
1	A	TGA	AA	AAG	A	ATATO	CGC	TT	TCT:	rcti	rGCA	TCT	OTA'	TTC	G 7	r TTT T	TCT	'TAT	TGCI	CAC	LAAC	
	- п	יאכי	TTT	ттс	T-	TATAC	GCG1	'AA'	AGA/	AGA	CGT-	AG/	YTAC	AAG	E-1	\A.A.A.	AGA	ATA.	ACGA	ATG	"T'TG	
-23	M	I	C	K	N	I	A	F	L	L	A	S	M	F	V	F	S	I	A	T	N	
				~~		TATA	~~ » (יחעב	CAC	ראר	בתככ	ccc	ZAGC	TCC	C I	rgrco	CGCC	CTC	TGT	3GG(CGAT	1
63	L G	CA.	'AC	CCI	i G	TATA	CAC	איייי מיייי	CTC	CCAC	2200	GGG	TTCC	AGG	G A	ACAGO	GCG	GAG	ACAG	ccc	CTA	
		:GT7	41G	N N	1C	I	0	M	Tr.	001	S	P	s	s	L	s	A	s	v	G	D	
- 3	5 F		ĭ	A	ט	_	Q	11	•	×	_	•	_	_								
101	. ,	·CC(<u> </u>	ארכ	٦Δ	TCAC	CTG	CAG	GTC	AAG'	rcaa	AG	CTT	GTA	AC 2	ATGG:	TAT	AGG	TAAC	CAC	TAT	
12.	L 27	יככי יכרו	CAC	TGC	3T	AGTG	GAC	GTC	CAG	TTC	AGTT	TC	GAA"	CA1	rg '	TACC	ATA'	TCC	ACG	ATG	CATA	
11				T			С				Q		L	V	Н	G	I	G	N	T	Y	
_	_																					_
18:	1 7	TA)	CAC	TG	ЗT	ATCA	ACA	GAA	ACC	AGG	AAAA	GC'	TCC	SAA	AC	TACT	GAT'	TTA	CAA	AGT.	ATCC	-
	2	\AT	GTC	AC	CA	TAGT	TGT	CTT	TGG	TCC'	TTTT	CG.	AGG	CTT	rg	ATGA	CTA.	AAT	GTT"	TCA	TAGO	š
3	8 1	ن	H	W	Y	Q	Q	K	P	G	K	A	P	K	L	L	I	Y	K	V	5	
															~~	ommo.	mcc	C 3 C	CCN	M TM	CACT	r
24	1 2	TAA	CGA	TTC	CT	CTGG	AGT	CCC	TTC	TCG	CTTC	TC	TGG	ATC	CG CC	GTTC	1.GG	CMC	CCT	<i>Y Y Y</i>	CAC:	<u>.</u>
		ГŢА	GC:	'AA	GA.	GACC	TCA	GGG.	AAG	AGC	GAAG	_AG	ACC.	<u>I'AG(</u>	<u> </u>	<u>CAAG</u> S	ACC G		CCI	F	T T	•
5	8 1	N.	R	F	S	G	V	Þ	S	R	r	5	G	5	G	5	G	-		•	•	
				7 % ED/	~ n	GCAG	·m~m	CCA	ccc	יאכא	ACAC	фф	ירפר	AAC	TT	ΑΤΤΑ	CTG	TTC	ACA	GAG	TAC	Г
30	1 (CTG	ACC	ינאנ	CM CM	CGTC	ACD	CCT	CGG	.תטת יייטתי	ਸ਼ੑੑੑੑਸ਼ੑੑੑੑੑਫ਼ ਗ਼ੑੑੑੑੵਜ਼ੑਜ਼ੑਜ਼ੑਜ਼	AA	GCG	TTG.	AA	TAAT	GAC	AAG	TGT	CTC	ATG	A
7				I			T.	0	P	E	D	F	A	T	Y	Y	С	s	Q	S	T	
,	•	ם	•	_		7	_	*	_	_	_											
36	1	CAT	GT	CCC	GC	TCAC	GTT	TGG	ACA	\GGG	TACC	AA	GGT	GGA	GA	TCAA	ACG	AAC	TGT	rGGC	TGC	Α
		CTA	CAC	GGG	CG	AGTO	CAA	ACC	TGT	rccc	ATGG	TI	CCA	CCT	CT	AGTI	TGC	TTG	ACA	CCC	SACG	T
. 9	8	Н	٧	P	L	T	F	G	Q	G	${f T}$	K	V	E	I	K	R	T	V	A	A	
42	1	CCA	TC'	TGT	CT	TCAT	CTI	CCC	GCC	CATC	TGAT	' G?	AGCA	GTT	'GA	AATC	CTGC	SAAC	TGC	_TTTC	.1G1	y .
		GG'	'AG	ACA	GA.	AGTA	\GA2	\GGG	CGC	GTAC	ACTA	C	rcgi	CAA	CT	TTAC	JACC O	JITG m	ACC	AAN. O	38CA V	~
11	8.	P	S	V	F	I	F	P	P	S	D	Е	Q	L	K	3	G	•	. ^	5	٧	
						TGA		C M M	CITI	N EDC	ירא <i>ר</i> ז	G	NGG(ממיי	ΔC	ጥልሮ	AGTO	GAA	GG'	rgg	ATAA	'C
4.8	31	GTC	etg etg	CCT	GC CC	ACT	u v wa 7.1.575	7C.T.T.	CN	22 CC	-CMGr	ים יו	えいいい	CTT	רידיכ	ATG'	rca	CCTT	CC	ACC'	rat'i	`G
		CAC	CAC	GGA T	.CG	ACT.	M.	GAA F	. GA.	DAT	2G 1C 1	E.	A	K	v	0	W	K	V	D	N	
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E /	4 1	CC	יריי	CCA	ΔT	CGG	TAZ	ACTC	CC	AGG	AGAG	r G'	TCAC	CAGA	AGC	AGG	ACA	GCAA	GG	ACA	GCAC	CC
5.	± ±	CC	CV	GGT	איזי	GCC	CAT	rgag	GG	TCC	rctci	A C	AGT	GTC?	rcg	TCC	TGT	CGTI	CC	TGT	CGT	3G
11	5.8	A	T.	0	S	G	N	S	0	E	S	V	T	E	Q	D	S	K	D	S	T	
6	01	TA	CAG	CCI	CA	GCA	GCA	CCCI	' GA	CGC'	TGAG	C A	AAG	CAG	ACT	ACG	AGA	AACA	CA	AAG	TCT	AC
		AT	GTC	GGA	GI	CGT	CGT	GGGA	CT	GCG.	ACTC	3 T	TTC	GTC'	TGA	TGC	TCT	TTGT	r GT	TTC	AGA'	rg
1	78	Y	S	L	S	S	T	L	T	L	S	K	A	D	Y	E	K	H	K	· V	Y	
																			n		~~~	~ »
6	61	GC	CTG	CG	LAC	TCA	CCC	ATCA	GG	GCC	TGAG	C T	CGC	CCG'	TCA	CAA	AGA	GCT"	r CA	MCA	,GGG	GA CT
		CG	GAC	GC1	rTC	AGT	GGG'	TAGI	CC	CGG	ACTC	G A	GCG	GGC.	AGT ~	GTT	TCT	CGA	n Gi	167	ر در در	- 1
1	98	A	С	E	V	T	Н	Q	G	L	S	S	P	٧	'1	· K	. 5	r r	L	· r		
-				، نعاماً ا		CTG	አመጣ		ר א	,000	CC 2 C	G (י איזיערי	Cuc	GCC	ርጥል	(GT)	CGC	A AC	TAC	TCG	TA
7	21	GA	GIC	יעעי דדר	747(747(GAC	MIC MAC	CICI	L AC	.GCC	CCTC	د ر	TAC	CAC	CGC	GAT	CAT	'GCG'	т тс	ATC	AGC	ΑT
^	10			AA. O	(, GAC	170	GAGI	- 10				, , , , , ,									
4	ΤQ	ن	_	•				-			=10	2	2	11	R							
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anti-IL-8 6G4.2.5V19 Light Chain Amino Acid | Sequence of the humanized

ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG LHWYQQKPGK|APKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTIS\$LQPEDFATYYCSQST HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLMNFYPREAKVQWKVDN MKKNIAFLLASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTY

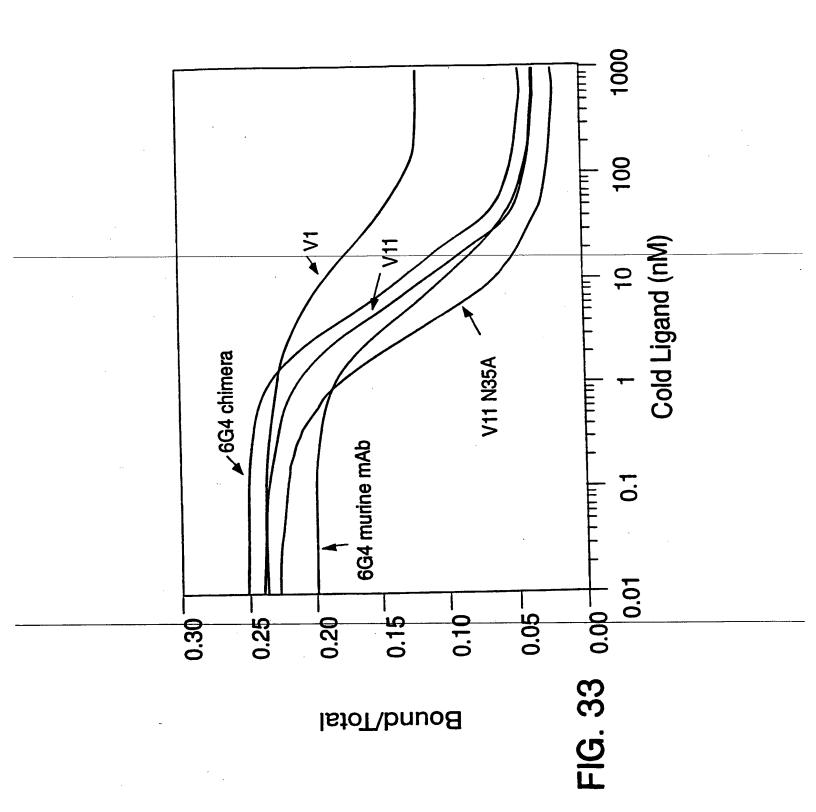
anti-IL-8 6G4.2.5V19 Heavy Chain Amino Acid Sequence of the humanized

WVKQAPGKGIJEWVGYIDPSNGETTYNQKFKGRFTLSRDNSKNTAYLQMNSLRAEDTAVYY CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKS†SGGTAALGCLVKDYF PEPVTVSWN\$GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG†QTYICNVNHKPSNTK MKKNIAFLLASMFVFSIATNAYAEVQLVESGGLVQPGGSLRLS¢AASGYSFSSHYMH VDKKVEPKS¢DKTHT

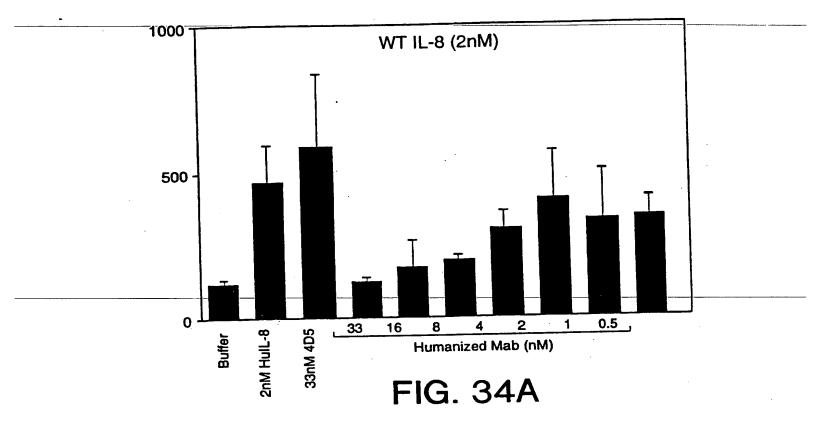
FIG. 31C

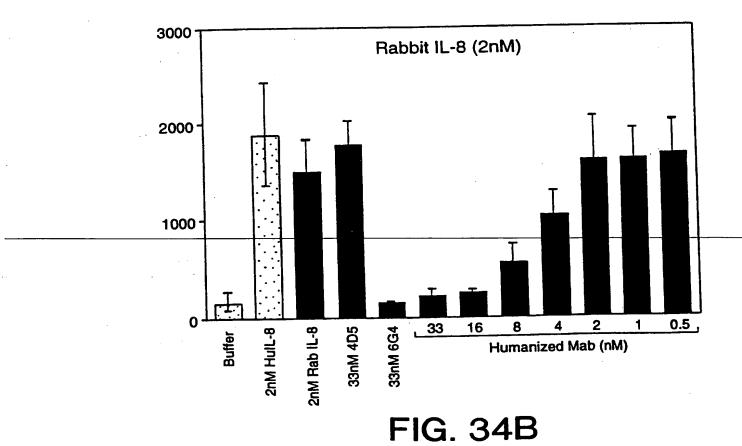


F16.32

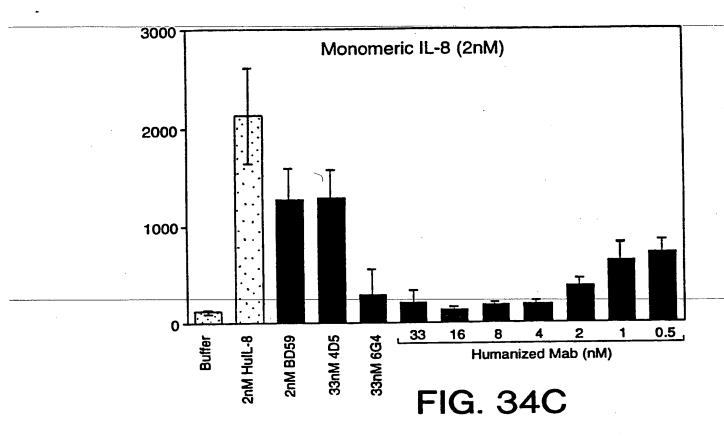


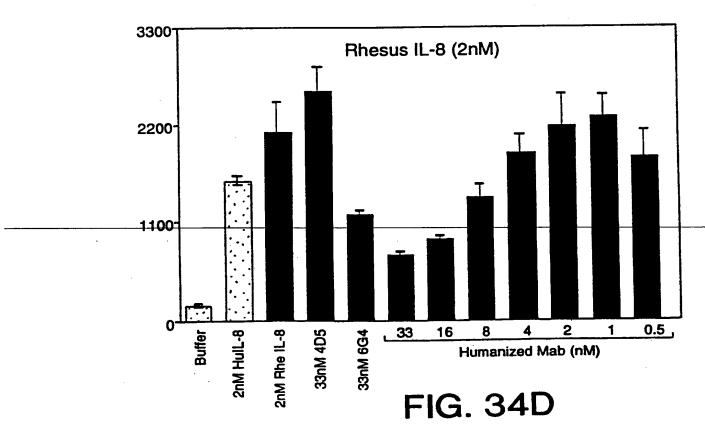
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anti-IL-8 6G4.2,5V11N35A Light Chain Amino Acid Sequence of the humanized

AL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN MKKNIAFLLASMFVRSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIG**A**TY LHWYQQKPGKAPKLIJIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST

Chain anti-IL-8 6G4.2,5V11N35A Heavy Amino Acid Sequence of the humanized

CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALT\$GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK WVRQAPGKGLEWVGYIDPSNGETTYNQKFKGRFTLSRDNSKNTAYLQMNSLRAEDTAVYY MKKNI AFLLASMFVFSIATNAYAEVQLVQSGGGLVQPGGSLRLSCAASGYSFSSHYMH VDKKVEPKSCDKTH! Amino Acid Sequence of the putative Pepsin Cleavage Site and GCN4 Leucine Zipper

CPPCPAPE<u>LL</u>GGRMKQLEDKVEELLSKNYHLENEVARLKKLVGER

FIG. 35

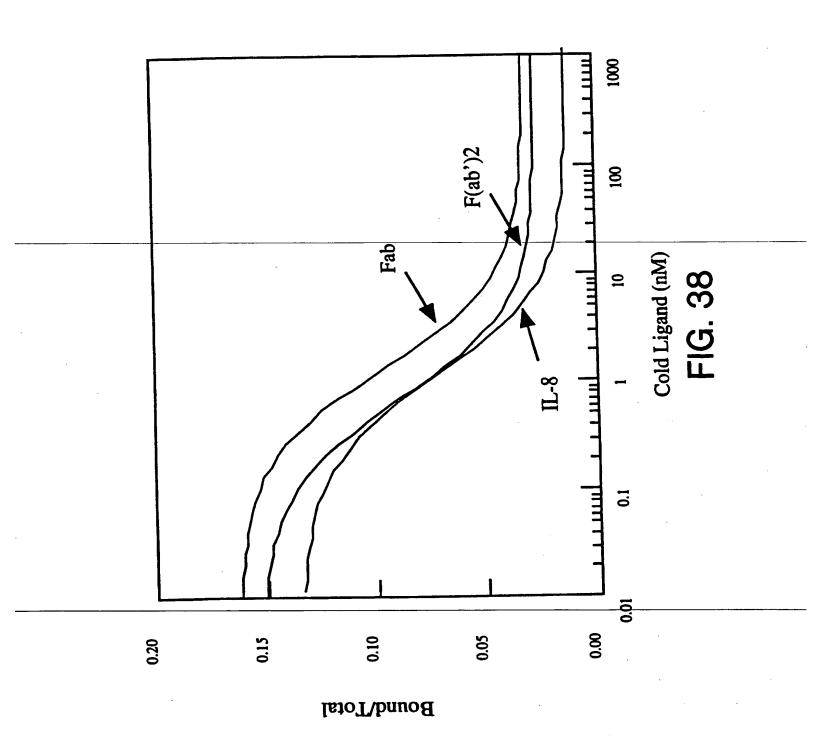
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٠	1	ATG	GAA	AAA	GA	ATATO	CGC	\mathbf{TT}	TCT?	CTI	rgca	TC:	OTAT	STTC	:G '	TTTTI	TC1	'A'I'	TGC	PACA	LAAC	
		TA	CTT'	rtt(CT	TATA	GCG'	raa	AGA	\GA/	ACGT	AG	ATA	CAAG	iC .	AAAA	AGA	ATA	ACG	ATGT	TTG	
	-23	M	K	K	N	I	A	F	L	L	Α	S	M	F	V	F	S	I	A	T	N	
	61	GC	ልጥል	CGC'	TG	ATAT	CCA	GAT	GAC	CAC	STCC	CC	GAG	CTCC	C	TGTCC	CGCC	CTC	TGT	GGG	CGAT	
		CC	יים איד	CCG	DC	TATA	GGT	CTA	CTG	GT(CAGG	GG	CTC	GAGG	3G	ACAGO	CGC	SAG	ACA	CCC	CTA	
	•	20	T.	GCG.	ח	T	0	М	T	0	S	P	S	S	L	Ş	A	S	V	G	D	
	- 3	A	1	A	ט	_	¥		•	×	_	_	_			•						
	404			~ > ~	C 3	mc » C	CTC	CAG	GTC:	ممدد	ממסת	AG	יייים איי	АСТР	\C	ATGGT	TAT!	AGG	TGC	TAC	TAT	
	121	AG	-C-1	CAC	CM	A CITICAL		CMC	CAC	ישנים	CTUT	TC	יממבי	יב איז דר איז	rG	TACC	יאדי	rcc	ACG	ATG	CATA	
		TC	CCA	ائی 11نی 	GT.	AGTG	GAC	216	CAG.		AGII	-	T.	17	u	G	т	G	A	T	Y	
	18	R	V	T	Τ	Т	C	<u>K</u>	<u> </u>	<u> </u>		٥										
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	181	TT	ACA	CTG	GT	ATCA	ACA	GAA	ACC	AGG	AAAA	GC	100	omm omm	4C	TACTO	3M 1 .	7 YW		WC 21	TACC	,
		AA	TGT	GAC	CA	TAGT	TGT	CTT	TGG'	rcc'	TTTT	CG.	AGG	C.I.I.1	IG -	ATGA	_TA4	AAT.	GII	ICM	INGG	
	38	<u>L</u>	H	W	Y	Q	Q	K	P	G	K	A	Р	K	Ъ	L	T	Y	4		_3	
	241	AA	TCG	ATT	CT	CTGG	AGT	CCC	TTC'	TCG	CTTC	TC	TGG	ATC	CG	GTTC'	rgg	GAC	GGA	TTT	CACT	
		TT	AGC	TAA	.GA	GACC	TCA	GGG	AAG	AGC	GAAG	AG	ACC	TAG	ЭC	CAAG	ACC	CTG	CCT	AAA	GTGA	
	58	N	R_	F	S	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	
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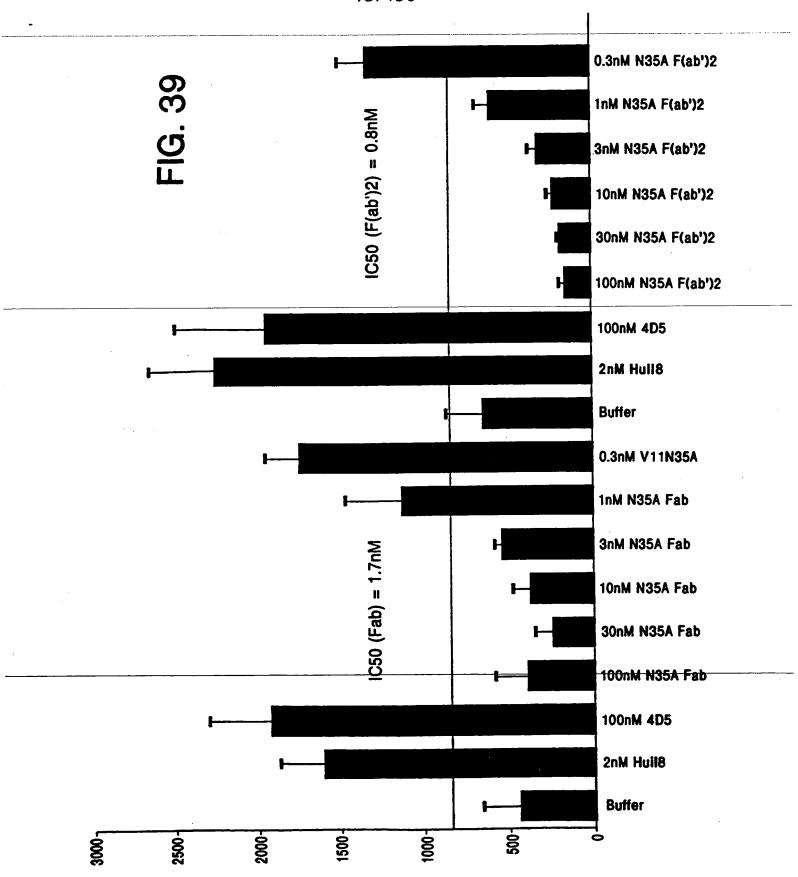
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1621 GAGGACAAGG TCGAAGAGCT ACTCTCCAAG AACTACCACC TAGAGAATGA AGTGGCAAGA CTCCTGTTCC AGCTTCTCGA TGAGAGGTTC TTGATGGTGG ATCTCTTACT TCACCGTTCT 248 E D K V E E L L S K N Y H L E N E V A R

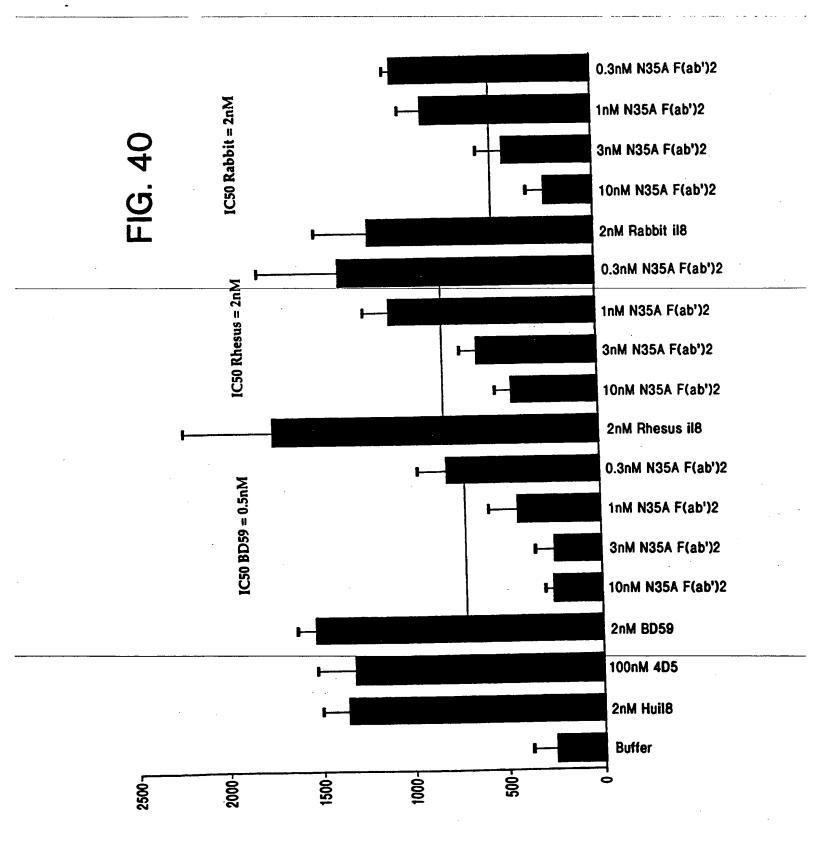
1681 CTCAAAAAGC TTGTCGGGGA GCGCTAA
GAGTTTTTCG AACAGCCCCT CGCGATT
268 L K K L V G E R O

FIG. 37B





SUBSTITUTE SHEET (RULE 26)



		30 7 730	
-			alui ssti saci hgiJii hgiAi/aspHi ecil36ii bspl286 bsiHKAi bmyi qi qi caGC
plei mboli taqi eari/ksp632i II hinfi GA AGAGTCGAAT	sau3AI mbol/ndell[dam-] dpn1[dam+] bcl1[dam-] rGAT CAGGTAGAGG	mbli ti ti tc ctcgtcagta ng gagcagtcat	ecoRI ta apoI GAATTC
mbo ear mboli aaaaagaaga	(E GTTGATT CAACTAA	fol sfan TTGAAGCA1	rmal mae! bfal mae!!!
alui hindili tru9! msel cac8! GTTGTTATT AAGCTTGCCC		I AAAGAAGTTA TTTCTTCAAT	tru9I mseI TTTTAATGTA
	hinpi hhai/cfoi GCGCAAAATG	thal fnudII/mvnI fnu4HI bsoFI bbvI maeII fnu4HI bstUI snaBI bsoFI bsh1236I bbvI hinPI bsaAI aluI hhaI/cfoI GAGCTGCTGC GCGATTACGT	antiosi TATA ATAGTCGCTT TGTTTTTATT TATCAGCGAA ACAAAATAA
ddeI bsrDI TCATTGCTGA	TCGCAATATG		pall III/eclXI ahdi/eaml1051 GACTT ATAGTCGCTT CTGAA TATCAGCGAA
nlaiii Aaatacagac atgaaaaatc Tttatgectg tacttttag	msli maeili bsrDi cgrca crgcaargcr gcagr gacgtracga	A CGACGATACG T GCTGCTATGC	eIII/ I / xma I / xma I / xma I / xma C C C G C C G C C G C C G C C C G C C G C C G C C G C C G C C G C C G C C G C C G C C C G C
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TTGGATAAGG AACCTATTCC	alui ndiii AGCTTTGGAG TCGAAACCTC	cac8I sfaNI CCCGATGCCA	uI III BII GCTGTCATAA CGACAGTATT
r Trgg	aluI hindiii R AGCT		alui pvuli nspbli nca gere
PflMI bsli TCTCCATACT TTGGATAAGG	bspMI hinpI hinpI hhal/cfoI mstI aviII/fspI hi caactGTGG CGCAGGTAGA	I I mnli 6I . CGAGGTAAAG	
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SUBSTITUTE SHEET (RULE 26)

TTTTCTATTG CTACAAACGC AAAAGATAAC GAIGTTTGCG F S I A T N A changed fr G toT ^	ddel nlaili alui rsai hindiil csp6i r CAAGTCAAG CTTAGTACAT A GTTCAGTTC GAATCATGTA S S Q S L V H
ANI ATC TATGTTCGTT TAG ATACAAGCAA S M F V Ducleotide was	bspMI scfI pstI pstI pstI pstI pstI pstI pspMI pstEII pphI psgI gGTCACCATC ACCTGCAGG CCAGTG TGGACGTCCC V T I T C R S
asp718 bamHI avaI npn1 acc651 alwI[dam-] mnl1 mnl1 401 TCGCTACCATC TATGTTCGTT TTTTCTATTG CTACAAACGC 401 TCGCTACCGG GGGATCCTCT CGAGGTTGAG GTGATTTAT GAAAAAGAT ATCGCATTTT TTCTTGCTACGTAG ATACAAGCAA AAAAGATAAC GATGTTTGCG AGCCATGGGC CCCTAGGAGA GCTCCAACTC CACTAAAATA CTTTTTCTTA TAGCGTAAAG AAGAACGTAG ATACAAGCAA AAAAGATAAC GATGTTTGCG AGCCATGGGC CCCTAGGAGA GCTCCAACTC CACTAAAATA CTTTTTCTTA TAGCGTAAAG AAGAACGTAG ATACAAGCAA AAAAGATAAC GATGTTTGCG AGCCATGGGC CCCTAGGAGA GCTCCAACTC CACTAAAATA CTTTTTCTTA TAGCGTAAAG AAGAACGTAG ATACAAGCAA AAAAGATAAC GATGTTTGCGATGGC CCCTAGGAGA GCTCAACACACACAAAACAAA	ssti saci hgiJII hgiAl/aspHI ecl136II bsp1286 bsiHKAI ri bmyl mnli aval alui acii cc GAGCTCCCTG TCGCCGATAG GGG CTCGAGGAC ACCCGCTATC p S S L S A S V G D R
asp718 bamHI avaI acc651 alwI[dam-] mn 401 TCGCTACCCG GGGATCCTCT CGA AGCCATGGGC CCCTAGGAGA GCT -23 a mutation was found t	bsm ecoRV tth1111/as 501 ATACGCTGAT ATCCAGATGA CCCAGTC TATGCGACTA TAGGTCTACT GGGTCAG

SUBSTITUTE SHEET (RULE 26)

xmal/pspAl smal scrFl ncil dsav cauli bsaJl

scrfi ncii mspi hpaii dsav

•		
FI [dcm-] pleI hinfI GGAGTCCCTT CCTCAGGGAA G V P S	rsal csp61 scal blaIII AGAGTACTCA TCTCATGAGT S T H	GCAGTTGAAA CGTCAACTTT Q L K
tfil bsmFI bpmL/gsuI[dcm-] clal/bspl06 pleI bspDI[dam-] hinfl A TCGATTCTCT GGAGT(T AGCTAAGAGA CCTCA(TACTGTTCAC ATGACAAGTG Y C S Q	CATCTGATGA GTAGACTACT S D E
hi taqi AAGTATCCA TTCATAGGT	CGCAACTTAT	acii mboli zarcececes racaagece
CTGATTTACA GACTAAATGT L I Y K	mboli bpual bbsi CAGAAGACTT GTCTTCTGAA	mboli bpual bbsi atcrecter ragacagaag s v F
TCCGAAACTA AGGCTTTGAT P K L	fnu4HI bsoFI bbvI scfI pstI bsgI AGTCTGCAGC TCAGACGTCG	ndell[dam-] fnu4HI mboll dam+] bsofl bbull bbull dam+] aAACGAACTG TGGCTGCACC ATCTGTCTTC TTTGCTTGAC ACCGACGTGG TAGACAGAAG K R T V A A P S V F F G C C A C C C C C C C C C C C C C C C C
rrfi rai corii sav alui stni alui cyi[dcm+] caggaaaagc GTCCTTTTCG	GACCATCAGC CTGGTAGTCG	~ \ \ \ \ \
000	ATTTCACTCT TAAAGTGAGA	
bsri ACACTGGTAT TGTGACCATA	I [dam-] I bsmFI BAGACCTGCC G G I D	styl bsaJI rsaI csp6I nlaIV kpnI hgiCI banI asp718 acc65I G T K
	mspi hpali bsli bsavi sau3Ai mbol/ndeli[dam-] dpnl[dam+] dpnl[dam+] alwl[dam-] nlalV bstrl/xholl bamHi alwl[dam-] bamHi alwl[dam-] cGATCCGGT TCTGGGA ACCTAGGCCA AGACCCT	maell ACGTTGGAC TGCAAACCTG T F G Q
GGTATAGGTG CTACGTATTE CCATATCCAC GATGCATAAA G I G A T Y L	CTCGCTTCTC GAGCGAAGAG	bsrBI acil bsmFI TGTCCGCTC ACAGGGCGAG V P L
601	701	801

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scrFI mvaI ecoRII dsaV bstNI bsaJI I apyI[dcm+] CCC AGGG	cac8I ACGC FGCG A	rmal mael bfal sau961 haeIII/palI nGCCCT
maeIII GGTAACI CCATTG	acci ca ACA AAGTCTACGC IGI TTCAGAIGCG K V Y A	I sfani gca togt cgt agca
mnli bsli ccrccaa ggaggti	AC GAGAAACACA .TG CTCTTTGTGT	
TGGATAAC ACCTATTG	SEPI SUL1021 TA AGCAGACTAC STT TCGTCTGATG K A D Y	
xmnI xmnI xmnI xmnI asp700 TCTGGAACTG CITCTGTTGT GTGCCTGCTG AATAACTTCT ATCCCAGAGA GGCCAAAGTA CAGTGGAAGG AGACCTTGAC GAGACAACA CAGGGACGAC TATTGAAGA TAGGGTCTCT CCGGTTTCAT GTCACCTTCC S G T A S V V C L L N N F Y P R E A K V Q W K V	celli/e blpi/bl il ddel cccrcacc cccrcacc	tru mse GTGTTP CACAA1
haelli/pall hael rsaf mnll csp6 (GA GGCCAAAGTA CT CCGGTTTCAT	fnu4BI bsoFI bbvI hga GC AGCACCCTGA CG TCGTGGGACT S T L T	aluI AAGAGCTTCA ACAGGGAGA TTCTCGAAGT TGTCCCTCT K S F N R G E
I m TATCCAGAGI	fnu4 bsoF ddeI EI mall bbvI A CAGCCTCAGC A F GTCGGAGTCG T	
xmnI cac8I asp700 GTGCCTGCTG AATAACTTCT ATCCCAGAGA CACGGACGAC TATTGAAGA TAGGGTCTCT C L L N N F Y P R E	sofi ACAGCACCTA (TGTCGTGGAT (S T Y	aspell III III II C CCCCTCACA C CCCCTCACA C CCCCCTCACA C CCCCCCACA C C CCCCCACA C C C C C
cac8I as	GACAGCAAGG CTGTCGTTCC D S K D	cac81 aluI astI sstI sacI hgiJII hgiAI/aspHI ec1136II bsp1286 bsiHKAI bmyI bmyI bmyI bmyI bmyI bmyI bau96I banII asu1 ddeI ec0109I/draII alwNI(dcm-) cAGG GCCTGAGCTC GCCC GTCC CGGACTCGAG CGGG
CTTCTGTTGT GAAGACACA S V V	III PACAGAGCAG STGTCTCGTC T E Q	cac8I aluI sstI sstI sacI hgiJII hgiJII hgiJII bgiJ286 bsp1286 ccl136II asul ddeI asul ddeI asul ddeI asul ddeI T B Q G L S S T B Q G L S S
xmbI asp700 rcrggaacrg c agaccrrgac g	maeili AGGAGAGTGT CACAGAGG GACAGCAAGG ACAGCACCTA TCCTCTCACA GTGTCTCGTC TGTCGTGGAT ESVIEQDSKD	cac8I aluI sstI sstI saci hgiJII hgiAI/aspHI ec1136II bsp1286 bsiHKAI bmyI bmyI bmyI bmyI bmyI bmyI bmyI bmy
10 901 13 2 P. T.	1001 A T	11011

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	TATGITCGIT TITICIAITG ATACAAGCAA AAAAGATAAC M F V F S I A		alwNI[dcm-] fnu4HI bsoFI	bbvi Tgigcagctt Ciggctactc Acacgicgaa gaccgaigag C A A S G Y S				
	II sfani TTCTTGCATC AAGAACGTAG L A S		aluI f	CCGTTTGTCC GGCAAACAGG R L S				
	mbo Gaaaaagaat atcgcatttc CITTTTCTTA TAGCGTAAAG K K N I A F L	scrFI mvaI ecoRII dsaV	mval fnu4HI ecoRII dsay bstNI hgiJII bstNI bsoFI bsaJI bmyI apyI[dcm+] bsaJI bmyI	5 2 2			14 41E	·
	hi : GTGATTTTAT CACTAAAATA		scrFI mval fnu4 ecoRII dsav bstNI bstNI bsoF apyI[dcm+]	acil hacilitic coccedence of the coccedence of t	scrFI ncil mspl hpaII dsaV	cauli bsli xmal/pspAl smal scrFl	dsav scrFI cauli mval bsli ecoRII	
	rmai maei hphi bfai hphi xbai mbli mnli AAGGGTATCT AGAGGTTGAG GTGATTTTAT TTCCCATAGA TCTCCAACTC CACTAAAATA		rma I mae I	Drai lui GCTAG CGATC L V			2 5 5 4	
			<u>6</u> 6	₩				
	rmal mael rsal bfal csp61 spel 1201 AGTACGCAAC TAGTCGTAAA TCATGCGTTG ATCAGCATTT		rsaI bs1WI/i thaI fnuDII/m bstUI bsh1236I	mlui csp61 mnL afliii ddei 1301 CTACAAACGC GTACGCTGAG GATGTTTGCG CATGCGACTC				
ř	H			77				

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		pall/draII
maeli bsaki TACGIATAAT T Y N	cac81 mnlr cac81 ddel drdi GCCTGCGTGC TGAGGACACT GCCGTCTATT CGGACGCACG ACTCCTGTGA CGGCAGATAA L R A E D T A V Y I	sau96I haeIII/palI sau96I nlalv hgiJII bmyI bsp120I bsp120I bsp120I bsp120I bsp120I bsp120I bsp120I bsp120I cool09I/draII rGGCCTCCA CCAAGGGCCC AGCCGGGG GTCCCGGG S A S T K G P 25chim2.fab2
snaBI hphi ATGGTGAAC TACCACTTTG	cac8I mnli cac8I ddel drdI GCCIGCGTGC TGAGGACACT CGGACGCACG ACTCCTGTGA	T TC SS
bsli sau3Al mbol/ndell[dam-] dpnl[dam-] alwl[dam-] alwl[dam-] ATATT GATCCTTCCA TATATA CTAGGAAGGT		maeIII bstEII scrFI mvaI ecoRII bsaJI asu dsav bseRI bstNI esp3I mnlI b aIV apyI dcm+1 bsmBI mnlI b aIV apyI dcm+1 bsmBI mnlI b T L V T V S S A S T seq right is from p6G425chim2.fab2
bsaJI dsaV avaI bstNI bsaJI bslI sau96I apy1[dcm+] nlaIV sau96I mbol/ haeIII/palI asuI ecol1091/draII haeIII/palI TCAGGCCCCG GGTAAGGGC TGGAATGGGT TGGATATATA AGTCCGGGC CCATTCCCG ACCTTACCA ACCTATATAAA	II II S CAGATGAACA G GCCTACTIGT	
dsav bstNI bslI apyl[dcm+] sau961 asuI ecool091/dralI I haeIII/palI AGGGCC TGGAATGGGT TCCCGG ACCTTACCCA	scfi psti bsgi bspMi AGCATACCTG	maell hinli/acyl ahali/bsaBl qi aatli GACGTC TGGGTCAAG CTGCCA ACCCCAGTTC D V W G Q G
bsaJI dsaV avaI bstNI bsaJI bslI sau96I apyI[dcm+ nlaIV sau96I haeIII/palI asuI asuI eco01091/draII haeIII/palI AGGCCCCG GGTAAGGCC TGGAAT TCCGGGG CCATTCCCG ACCTTA	I CCAAAAACAC GGTTTTTGTG	maell hinli/acyl ahali/bsaB taqi mboli aatli rr crrcGACGTC TGG tA GAAGCTGCAG ACC TARE D V W
	thal fuuDII/mvnI bstUI bsh1236I nruI cACTTTATCT CGCGACAACT GTGAAATAGA GCGCTGTTGA GGTTTTTGTG	maeIII hphi bsii mb ccctacaatg GrgacrgGTT GCGATGTTAC CACTGACCAA R Y N G D W F
sau961 avall asul nlaIV bsrI ACTGGGTCCG TGACCCAGGC	n TITATCI RAATAGA L S	h CTACAATG SATGTTAC Y N G
	haeIII/pall sau96I asul AGGGCGTTT CAC TCCCGGCAAA GTG	11 AGGGGATTAT TCCCCTAATA G D I
pleI hinfI taqI xhoI paeR7I avaI mae CTTCTCGAGT	CAAAAGTTCA GTTTTCAAGT Q K F K	m 1 ACTGTGCAAG TGACACGTTC 6 C A R
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hphI mspI hpaII cfr101/bsrFI bsaWI tth1111/aspI bs1I ageI maeIII AAGGACTACT TCCCCGAACC GGTGACGTG TTCCTGATGA AGGGGCTTGG CCACTGCCAC	ddel fnu4HI maeIII mnlI boot bsoFI maeIII mnlI boot ddel bsoFI hinfi ddel byl bstEII bmyl bpmI/gsuI[dcm-] ccracagrcc rcagactcr Acrccrcag caccacacac rgcacacacacacacacacacacacacacacacacacaca	hgiJII bsp1286 ndII bmyI maeIII banII grGACAAAC GAAAGTIGAG CCCAAATCTT GTGACAAAAC CTTTCAACTC GGGTTTAGAA CACTGTTTTG K V E P K S C D K T
scrFI mval scrFI mval scrFI mval ecoRII ecoRII ecoRII esoRII dsaV bstNI bstNI fnu4HI fnu4HI bsp1286 bsp1286 bsp1286 bsp1286 bsp1286 acii apyI[dcm+] bsiHKAI bsp1286 acii apyI[dcm+] bsiHKAI bsp1286 ccccGGGCACCCGGCCCGGCCCGGCCCGGCCCGGCCCGG	hgial/aspHI bsp1286 bsiHKAI mspI 81 scrFI bmyI ncil apaLI/snol dsaV alw441/snol caulI GTGCACACCT TCCCGGCTGT	tagi sali hinfi maeli gcaacgrgaa TCACAAGCCC AGCAACACCA AGGTCGACAA N N N N N N N N N N N N N N N N N N N
nlaIV hgiCI bani scrFI mval ecoRII dsaV mboII bstNI bseRI bpual bsaJI mnlI bbsI bsaJI mnlI bbsI bsaJI mnlI rAGCCAGAAG GGGACCGTG GGAGGGGTT S V F L A P S S K	hinpI hhal/cfoI hhal/cfoI nlarV narI kasI hinlI/acyI hgiCI haeII banI ddeI ahaII/bsaHI ns I TCGTGGAACT CAGGCGCCT GACCA AGCACCTTGA GTCCGCGGGA CTGGT	alul nlaIV fnu4HI hgiCI bsoFI banI bbvI bsp1286 bstXI bmyI 19(1 CCAGCAGCTT GGGCACCCAG ACCTACATCT GGTCGTCGAA CCGTGGGTC TGGATGTAGA 196 S S L G T Q T Y I C

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fuutHI bsoFI cac81 nialII nspl nspl nspl nspl nspl nspl nspl nsp			scrFI ncii ncii nspi hpaIi dsaV cauIi acii fnu4Hi bsoFI ACGCTCGGTT GCCGCCGGC	nlaIV hgiCI banI rGCTAA CGCAGTCAGG CACCGTGTAT GAAATCTAAC ACGATT GCGTCAGTCC GTGGCACATA CTTTAGATTG
H #		fnu4HI bsoFI hacII/palI mcI eagl/xmaIII/e eaeI cfII bsiEI notI fnu4HI s6 aciI aciI aciI aciI aciCAGCACCAGA ACTGCTGGGC GGCGCCATGA GTCGTGGTCT TGACGACCC CCGGCGTACT A P E L L G G R M K een antibody and leucine zipper	sphi ddei nlaii celli/espi blpi/bpull0 hinPi nspi hhal/cfol hindili eco47111 cac81 CAAAAAGCTT GTCGGGGAG GCTAAGCATG GTTTTTCGAA CAGCCCTCG CGATTGTAC	taqi hindili clai/bspl06 tru9i clai/bspl06 tru9i tui bspDi[dam-] msel acii msel sc TIATCALCGA TAAGCTTTAA TGCGGTAGTT TATCACAGTT AAATT sc ATAACAAGT ATTCGAAATT ACGCCATCAA ATAGTGTCAA TTTAA
		n1 nsp nsp rcacaca agrerer	GAGAATC CTCTTAC	

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haeIII/palI sau96I scrFI sil spl mnli joi hpaII ecoRV cauII aciI ACCGGGCCT CTTGCGGGAT ATCGTCCATT TG ACGCCCGGA GAACGCCCTA TAGCAGGTAA	fnu4HI hgiAI/aspHI bsoFI bsp1286 acii haeIII/ bsiHKAI mcrI eaeI bmyI bsiEI cfrI GTTCTCGGA GCACTGTCCG ACCGCTTTGG	mnll sau3Ai mbol/ndeII[dam-] dpnI[dam+] dpfnI[dam-] alwI[dam-] nlaIV bstXI/xhoII hgaI bamHI mspI bamHI alwI[dam-] hpaII sfaNI crecacacc Greeres recreace cesaceare crecacacc Cacacacare
hael sau961 sau961 scrFI ncil scrFI csp61 hpaII nsp1 dsav bhpaII cfI101/bsrFI asuI argccGGTAC rGCCGGGC	hinPI hhal/cfoI mstI bsll aviII/fspI TATGCGCACC C	I[dam-] -] -shire psli
AGGCAT AGGCITGGIT	sfani ATGCGTTG ATGCAATTTC TACGCAAC TACGTTAAAG	sau3AI mbol/ndeII[dam-] dpnI[dam+] dpnII[dam-] thaI fnuDII/mvnI bstUI nlaIII taqI bsh1236I ATCGACTACG CGATCATGGC GACCAC
sfaNI scrFI mvaI mvaI ecoRII dsaV bstNI bsaJI hphI apyI{dcm [†] maeIII fokI cGTC ACCCTGGATG	hinpi hhal/cfol rmal mael maelII flu4HI haelI bsorI eco47III bbvI bfaI cac8I cac8I cGCCAGTCAC TATGCGTGC TGCTAGCGCT ATA	nlaIV TGGAGCCACT ACCTCGGTGA
mnli bsad cfoi foki CA TCGTCATCCT GT AGCAGTAGGA	maeIII sfaNI bsII c GCAT CGCCAGTCAC TATGG	acii fnu4Hi bsoFi acii bsri cac8i CCGCCGCCCA GTCCTGCTCG GGCGGCGGT CAGGATGA
hinPI hhal/ TAGGGGT	sfani 2401 CCGACAGCAT	acil fnu4HI bsoFI acil bsr1 2501 CGGCGGCGA

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	pall	
hgiJII bsp1286 bmyI banII u3AI cac8I ol/ndeII[dam-] hgiJII haeII nI[dam+] bsp1286 eco47III nII[dam-] banII nlaIII ATCGGGCTCG CCACTTCGG CTCATGAGCG	fnu4HI bsoFI hgiAI/aspHI acil bsp1286 fnu4HI bsiHKAI bsoFI bmyI haeIII/palI GCGCCGCCG TGCTCAACG	hpall bsawi bsri alui bsli ACCCAGTCAG CTCCTTCCGG TGGGTCAGTC GAGGAAGGCC
hgiJII bsp1286 bmyI banII nu3AI cac8I ooI/ndeII[dam+] onI[dam+] aTCGGGCTCG		AGAGCCTTCA TCTCGGAAGT
mbo CTACCCTT		sfani c Gargecerre g craegegaad
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hinPI hhal/cfoI nlalV narI kasI hinll/acyI hgiCI haeII banI ahaII/bsaHI GGCG CCTATATCGC		G CATANGGGAG IC GTATTCCCTC
hinpi hinpi hai/ nari kasi kasi haili/ haeri bani ahali/ il cac81	scrFI ncii nspi hpali dsal dsaV bsli cauli sau961 haelli/l nlaIV eael haell/pall asuI bsaJI bsaJI cGC CCGTGGCCGG	plei hinfi T GCAGGAGTCG A CGTCCTCAGC
cfol acyl bsaHI acil CACAGGTGCG	dsa sau961 bs1 nlaIV haeIII/F asuI bs8 eco01091/ cac81 bs11 GTGGCAGGCC CCG	I econi beli GCTTCCTAR CGAAGGATT
hinpi hhai/ hhai/ nlaiv nari kasi hyili/ hgili sgrai sgrai il hpaii hphi ahaii/ hghi ahaii/ hghi ahaii/	scrF] ncii nspi hpai] dsal dsav bsli cauli sau961 haeIII/ nlaIV eaeI haeIII/palI asuI bsaJI bsaJI ecol1091/draII cac8I bsli cfrI cacGCCCCGGGCCCGG	fnu4HI bsofi ecoNI pleI bstI bbvI bslI hinfI cracregect gcirccraar gcaggacteg Gargaccega cgaaggarra cgrccrcage
		mnli bsli ccrcaaccra ggagtrggar
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fnutHI msp1 himp! nae1 hae11 cfr101/bsrF1 cac81 eco47111 hialy bsoF1 hydror bsoF1 hydror baor ban hydrofol carocaacc grocogcage gerergeger artrogage gracerrag carecrore acgeccore gracers ranadecoge gracerrag carecrore acgeccore gracers ranadecoge gracerrag carecrore acgeccore gracers recaaarer recacger cacroacc recaract acil hinfi cac81 mn1 bsr11 bsr11 bsr11 th11 trecaaarer recacger gracerrag accordere agerraga acgracerraga acgracerraga accordere agerraga acgracerraga accordere angerraga acgracerraga accordere angerraga acgracerraga acgracerraga acgracerraga th13361 bsh13361 mn11 hae1 bsh13361 mn11 bsh13361 foth hae1 bsh13361 gargaccrace accacccare gcaracacca caracaccrac accacccare gcaracacca caracaccare accacaccare gcaracacca caracaccare	
mboll bpual bpual bbal crtargacrg rcrtcrtrar gaaracrgac agaagaara ni[dam+] nacil nacil naci cfri naci naci naci naci cfri naci naci naci cfri naci cfri naci cfri naci naci cfri naci c	FIG. 41K
acil thai funDII/mvnI bstUI nlailI bsh1236I hlnPI bcgI bcgI bscFI hhal/cfoI bscGGGGGGGGGGCTT thai funDII/mvnI bscGGGGGGGGGGTT thai funDII/mvnI bstUI acil bsh1236I sau sau96I bpml/gsu[[dcm-] dpu avali bpml/gsu[[dcm-] dpu asuI acil bpml/gsu[[dcm-] dpu bsh1236I sau bsh1236I sau bstUI cacGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	

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/aspBI B6 A1 nlaIII nlaIII ACATGGAACG GGTTGGCATG	fnu4HI bsoFI acil nspI mnlI hpaII nlaIV naeI hgiCI cfr101/bsrFI cac8I banI TGGAAGCCGC CGCCACCTCG
aJI aJI ca crcGC GAGCCG	I/palI taqI taqI ACC TCGACCTGAA rGG AGCTGGACTT
aci fnu4 bsof bgl1 TTTATGCCG	haell sau961 scrFl ncil mspi hpail dsav 7 asul ccull GGCCCGG
sau96I avalI bsrl sau3AI asuI mbol/ndeII[dam-] dpnI[dam+] nspBi dpnII[dam-] cGATCACTGG ACCGCT	thai thai thai fubli/mvni fuubli/mvni bstUi bstUi bsh1236i bsh1236i nlaiv mnli acii hgai acii nlaili crrcrcccccc rrccrcccc Grccrcccccccccccc
fnu4HI bsoFI acil thal thal fnuDII/mvnI bstUI cac8I sau3AI bsh1236I mboI/ndeII[dam-] dpnI[dam+] dpnI[dam+] cacGGGGGGGGATGG TCC	fnu4HI bsoFI hinPi hhal/cfoI nlaIV narI kasI hinl1/acyI hgiCI haelI banI aciI ahalI/bsaHI craacatccg cgcGGGGATA TGGAACAGAC
	fnu4HI bsoFI acil thal fnuDII/mvDI bstUI cac8! sau3AI bstUI cac8! sau3AI bsh1236I dpnI[dam-] dpnI[dam-] dpnI[dam-] dpnI[dam-] dpnI[dam-] dpnI[dam-] cacBraccrcc GCCTCTTACC ACCTACTT CGATCACTC TGGCGACTACTT CGATGCACT CTACTTGC CCACCGTAC CTAGCGACG CCGAGATGA GCTGACTACT CGATGCACT CGATGCCCT CTAGCGACG CCGAGATGA GCTAGCACTACT CGATGCACTAC CTACCGTAC acil mbol/ndeII[dam-] bsau3AI bsh1236I mbol/ndeII[dam-] bsau3AI bsh1236I cac8I bayI cac8I nlaIII nlaIII acil dpnI[dam-] bsau3AI bsh1236 cac8I nlaIII nlaIII dpnII(dam-) cacBraccacacacacacacacacacacacacacacacac

		(D.C.) H&H	Appendix and the second
hgal thai acil fnuDII/mvnI bstUI bsh1236I rcGCGTCCGC	I cac8I	drall acil GCTAGGCTGG CGATCCGACC nlallI ACAACATGAA	
AACATATCCA :		ecool091/drall caull.bfal ac TGAGGACCCG GCTAG ACTCCTGGGC CGATC ddel GACCTGAGCA ACAA(CTGGACTCGT TGTT(
styl bsaji ccctrggcag A	s I[dam-] nla] ava: asu]	mnll GACAGCA GACAGCA ACGTCTGC	
	sau3AI mboI/ndeI [dam+] I[dam-] hgiAI/aspHI bsp1286	bsine CCGTG (NGCAC (NGCAC (SOFI SOFI BVI HI I	
hinPI hhal/cfol aviII/fspl II ccccaaACcaA	P H S S	aviii/fspi lasi ssi bmyi srcc cardarc cacc cardarc cacc cardarc bso bbv fnu4ffi bsoFi bbvI cccrc accacc	
hine hhaj nsti avili bsmi ACTGTGAATG C	i I I I I I I I I I I I I I I I I I I I	ali mali caccecacc greccacce greccacce eli greangeceac	FIG. 41M
hinPI hhal/cfol mstl pflMi avill/fspl acil bsml bsli cTTGCGGAGA ACTGTGAATG CGCAAACCAA	haell/hael/hael/hael/hael/rrricorlicorlicorlicorlicorlicorlicorlic	ppurat nlary cfri ecool091/dr GGGTCCTGGC CCCAGGACCG cac81 al ubli/mvni tt13361 mad GCGAGCGAAC	
		hhal/cfol fnu4H1 1361 aval bsoF1 [sfaN1 bbv1 5GCGCATCTC GGGCAGGGTT 5CGCGTAGAG CCCGTCGCAA 5CGCGTAGAG CCCGTCGCAA hinf! bs hinf! bs hinf! bs	
I BlaIV AGAATTGGAG CCAATCAATT TCTTAACCTC GGTTAGTTAA	fnu4HI thai hinPi bsoFI fnuDII/mvbI	FI cac8I hhal/cfoI I acil bshl236I avaI I[dcm-] acil sfaNI AGCCGCACGC GCCGCATCTC GC TCGGCGTGCG CCGCGTAGAG CC TCGGCGTGCG CCGCGTAGAG CC TTACTGGTTA GCAGAATGAA T	
	fnu4H1 thai hi fnu4HI bsoFI bsoFI fnuDII/	bsofi cac8i hhal/cfoi bbvi acii bsh1236i aval bbvi acii bsh1236i aval carcrccac acccccacgc gccccarcrc gracacgrc rcgccgrcc ccccgracac bsri bsri tfi	
hphi tfii pfli hinfi bsll ctaacggatt CaccactcCa	·	bsoFI bbvI bbvI carcrccagc ag gradaggrcg rc	
0 8 C C C C C C C C C C C C C C C C C C		3601	and poor supplementation on the supplementation of the supplementati

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HI T Cacgarace	mbli TTTACCCTCA AAATGGGAGT	apol bsli AGAAATTCCC TCTTTAAGGG
fnu4 bsof bbvI sfaNI skI cracca	bsrI acii cccccagtTG GGCGGTCAAC	nlaiii Gtatcattac ccccatgaac Catagtaatg ggggtacttg
u3AI ol/ndeII[(an[[dam-]] il/xhoII ([dam-]] saBI[[dam-]] saBI[[dam-]] Arcrccarc	acil foki sau96i sfaNi nlaIV acil avali fnu4Hi asul bsoFi CTGGTCCCGC CGCATCCATA GACCAGGCG GCGTAGGTAT	
bs mspi hpaj bspki bspki bspki acci:		foki sfani grarcereng carcerere cetteares catageacre graggaga geaagfage
acil thai thai fuuDil/mvni hinPi bstui hhai/cfoi bsh1236i haeli msli rggaaacgc carcacaca	G ACTAAAAGA	foki sfani gratceteag catcetete catageaere graggagaa FIG. 41N
acil thai fuuDil/mvni hinPi bstui hhai/ bsh1236i haeli cGCG GAAGTCAGCG C	ddeI F TGACCCTGAG A ACTGGGACTC	
	cac81 hinPI hhal/cfoI naeII aco47III A GCGCTGCAT	maeIII A TCAGTAACCC T AGTCATIGGG
TCGTAAAGTC	tru91 } mseI TATTAACGA	nspi scrfi ncii mspi hpaii dsav nlaiii cauii maeiii nspii GTAACCGGC ATGTTCATCA
mboli bpual bbsi regictices ittecetett	CCTACATCTG	
	CTGTGGAACA	bsli maeli psp14061 1 caacgrrcca Grrgcaaggr
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I tru91 msel bpmI/gsul[dcm-] ratcagaagc cagacattaa cgcTtcTgGa GaaaCtCAAC ATAGTCTTCG GTCTGTAATT GCGAAGACCT CTTTGAGTTG	fnu4HI thal bsoFl fnuDII/mvnI aluI bstUI pvuII hinPI fnu4HI thaI bsoFl fnuDII/mvnI bcgI bstUI bbvI mnlI bsh1236I bbvI msh1236I hphI cccc GCGCTCGC GCGTTCGGT GATGACGTG GCGCT CGACGGAGCC CCCAAGCCA CTACTGCCAC	hgal thal fnuDII/mvnI bstUI acil bsh12361 hinPI nspBII hhaI/cfoI hdaI acil cccccccc ccccccccccccccccccccccccccc	
cac8I sau96I tru9I haeIII/palI mseI asuI aciI bslI nlaIII aciI CCTTTAACA TGCCCGGCGAA ATAGTCTTCG	xmnI xmnI tfil hinfi asp700 msli alul acil bbv1 tGTGAATCGC TTCACGACCA CGCTGATGACGCT ACACTTAGCG AAGTGCTGGT GCGCTGATACCCGCT	scrFI ncii ncii nspi hpali sfaNi foki dsav drdi acii cauri drdi acii cauri GCGTCACAGC TTGTCTGTAA GCGGATGCCG GGAGCAGACA GCCAGTGTCG AACAGACAT CGCCTACGGC CCTCGTGTGT	
-d e.	ប ២	esp31 bsmB1 bsmB1 msp1 scrF1 ncil dsav cauli il bsl1 CTCCGGAGA GAGGCCTCT	
sfani mbli maeili 4101 CCTTACACG AGGCATCAAG TGACCAAACA	acil thai thai fuuDil/mvai bstUi bsh1236i alui hgai foki crcGACCTGC GCGATGAACA	fnu4HI bsoFI bbvI nali napli alui 4301 AAACCTCTG ACCATGCAG C TTTTGGAGAC TGTGTACGTC G	

hgial/aspHI bsp1286 bsihKal 4HI ddel bmyl ndel FI csp6I alw441/snol G CATCAGAGCA GATTGTACTG AGAGTGCACC	hinPI hhal/cfol fnu4HI pleI bsoFI mcrI hinfI bbvI bsiEI GCTCACTGAC TCGCTGCGCT CGGTGTTCG	DIBILI DESLI DEPL CACEL DEPL HACILI/PALI AFILII HACI GGAAGAACA TGTGAGCAAA CCTTTCTTGT ACACTGTTT TCCGGTCGTT	hgai drdi taqi ACAAAAATCG ACGCTCAAGT CAGAGGTGGC TGTTTTAGC TGCGAGTTCA GTCTCCACCG
maeII fnu4H maeIII bst11071 tru91 bsoFI h1111/aspI acil acci bsrI msel acil cccaGC ACGTAGCGAT AGCGGAGTGT ATACTGGCTT AACTATGCGG	mboli earl/ksp6321 sapl hinPI scil haeli acil mnli TAAGGAGAAA ATACCGCATC AGGCGTCTT CGCTTCCTC ATTCCTCTT TATGGCGTAG TCCGCGAGAA GGCGAAGGAG	tfil hinfl GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA CCGCCATTAT GCCAATAGGT GTCTTAGTCC CCTATTGCGT	fnuDII/mvnI 12361 acii fnu4HI bsori cac8I haeili/pali cgcccctrc crgcccccr gacgagarc cgcccctac cacgccana aggrarcca gccgcgaga crgcrcgrag
fnu4HI bsoFI bbvI hinPI nlaIII hhaI/cfoI tt 4401 CGGTGTCGG GGCGCAGCCA TGF	acil sfani 4501 ATATGCGGTG TGAAATACCG CACAGATGCG	fnu4HI bsoFI acil fnu4HI acil bsoFI bsrBI bbvI cac8I 4601 GCTGCGGCGA GCGTATCAG CTCACTCAAA CGACGCCGCT CGCCATAGTC GAGTGAGTTT	scrFI thal fn mval botul ecoRII bstUI dsav bshl2361 acl apyl[dcm+] fnu4 haeIII/pall bsoF haeII back dGCCG A701 AAGGCCAGGA ACCGTAAAAA GGCCG TTCCGGTCCT TGGCATTTT CCGGC

acil mspl fuutii hpali bsoFl bsaWi crGGGACGCG CTTACCGGAT ACCTGTCCCC crGGACGCG CTACCGGAT ACCTGTCCCC crGGACGCG CTACCGGAT TGGACAGGCG crGGACGCG CTACCCGAT TGGACAGGCG bspl286 bspl2866 bspl28666 bspl286666 bspl2866666 bspl2866666 bspl28666666 bspl28666666 bspl286666666 bspl2866666666666 bspl28666666666666666666666666666666666666
acil msi fnu4H) bsoFI GACCTGCG (CTGGGACGC (TGAATAGCG rmai maei bfai CACTAGAAGG
acil ms bsoFI bsoFI GACCCGGCG CGGGAGGT TGATAGCGG TGATAGCGG CGGGAGGT CACTATCGCC TGGGAGGT CACTATCGCC GTGATATCGCC GTGATCTTCC GTGATCTTCC
bell crecretrec gaggacaagg gaggacaagg ccacarccag ccacarccag ccacarccag carrcrerec arracgccra acracgccra rgargccgar
scfi ddel besli belli besli cgcacacc crcaccaca gagacacca agacacaca gagacacaca agacacacac
BCIFI BCORII BAINE BAJI ALUI MINI CC TGGAAGCTCC CT GG ACCTTCGAGG GA GG ACCTTCGAGG GA GG ACCTTCGAGG TA GT GCGCATCCA TA GT GCGACATCCA TA FIGT BAGA GTCTTGAAG TG CCT CAAGAACTT CA CCT CAAGAACTT CAA CCT CAACTT CAACTT CAACTT CAA CCT CAACTT CAACTT CAACTT CAA CCT CAACTT C
scr al eco av tul apy alul alul AGGGGG AGGGGG AGCTCA TCCACTCA CCATTGA CCATTGA CCATTGA CCATTGA GATGTCT
scrfi mvai mvai dsav bstNi apyl(d ninPi ninPi scgctTTC cGCGAAG GCGCTTC GCGCATC I inPi ninPi acti
AGGACTATA A TCCTGATATT 1 TCGGGAAGCG 1 AGCCCTTCGC 2 AGCCCTTCGC 2 AGCCCTCGC 2 AGCCCGACGC GCTCGCC TCGGCCGGCGCGCGCGGCGGGCGGGGGGGG
2 00 1 1 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0

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fnu4HI bsoFI bbvI cac8I TTTTGTTG CAGCAGCAG	tru9I nlaIII mseI rcaI ACGTTAAGG ATTTTGGTCA TGCAATTCC TAAACCAGT	maeIII TAAACTTGGT CTGACAGTTA ATTTGAACCA GACTGTCAAT	mbli AGATAACTAC GATACGGGAG TCTATTGATG CTATGCCCTC
acii Agcegtett TCGCCACCAA	ACGAAAACTC TGCTTTTGAG	TATATATGAG	/eam1105I CCCGTCGTGT GGGCAGCACA
mspI hpaII sau3AI mbol/ndeII[dam-] dpnI[dam+] dpnI[dam-] aluI alwI[dam-] aciI actITTC TCACCATG AGAACTAGC CGITIGITIG GIGGGAA	sau3AI sau3AI mboI/ndeII[dam-] mboI/ndeII[dam-] oI/ndeII[dam-] dpnI[dam+] oI/ldem+] dpnI[dam+] onI[dam+] dpnI[dam+] oI/xhoII alwI[dam-] II/xhoII alwI[dam-] AFCTCAAGAA GATCCTTTGA TCTTTTCTAC GGGTCTGAC GCTCAGTGAT TAGAGTTCTT CTAGGAAACT AGAAAAGATG CCCCAGACTG CGAGTCACCT	sau3AI mbol/ndell[dam-] rmaI hphi dpnI[dam+] l[dam-] mseI dam+] dpnI[dam-] tru9I cam-] alwI[dam-] tru9I cam-] bfaI ahaIII/draI ahaIII/draI pAGTGGATCT AGGAAATTT AATTTTACT TCAAATTTA GTTAGATTTC	TV sau3AI pleI pleI pleI plofi phot/dam+]
maeili eco571 bsri 5201 TCTGCTGAAG CCAGITACCT TGGGA AGACGACTTC GGTCAATGGA AGCCT	hinpi sau3AI mbo: hhal/cfoi mboi/ndeII[dam+] thal dpnI[dam+] fnuDII/mvnI dpnII[dam-] bstUI bstII/xhoII bsh1236I alwI[dam-] b 5301 ATTACGCGCA GAAAAAAGG ATCTCAAGAA TAATGCGCGI CTTTTTTCC TAGAGITCTT	mbolidam-1 sau3AI maeI mbolidam-1 sau3AI maeI mbol/ndeII[dam-1 dpnI[dam+1] dp dpnII[dam+1] dp dpnII[dam-1] al bstYI/xhoII bst alwI[dam-1] bfaI stOI TGAGATTATC AAAAAGGATC TTCACCTAGA	nlalv hgidi tru9I bani msel mnli 5501 CCATGCTTA ATCAGTGAGG CACC GGTTACGAAT TAGTCACTCC GTGG

WO 98/37200			PCT/US98/03337
VVO 36.6.2.200	68 / 1	136	
fol		dam-]	1
haeIII/palI sau961 hinPI asuI hhaI/cfoI AGGGCCGAGC TCCCGCTCG	maell hinpl hhal/cfol mstl psp14061 avill/fspl rGGGAAGGT	sau3Al mbol/ndeIl[dam-] sau3Al dpn1[dam+] dpn1[dam+] dpnII[dam-] dpn1[dam+] nlaIII nlaIII dpnII[dam-] maeIII alwI[dam-] CAACGATCAA GGCGAGTTAC AYGATCCCCC GTTGCTAGTT CCGCTCAATG TACTAGGGGG	mbol/ndell[dam-] acii fnu4Hi dpn1[dam-] bsofi eael haelll/pali nlaili bsofi eael msli bsiEl cfrices actraces restrance cecescace restrance cecescace restrance cecescace restrance cecescace restrance restrance cecescace restrance restran
mspI hpaII bglI cac8I \GCCAGCCGGA	tru9I bsrI mseI TAGTTCGCCA GTTAATAGTT ATCAAGCGGT CAATTATCAA	sau3AI mbol/ndeII[dam-] dpnI[dam+] maeIII GATCAA GGCGAGTTAC A	fnu4 nlaIII bsof mslI bbvJ rCAT GGTTATGGCA
G CAATAAACCA C GITATIIGGI			nlal msli gt tatcactcat
bpmI/gsuI[dcm-] .1 .1/bsrFI laIV .CCCA GATITAICAG	scrfi ncil mspi hpali rmal dsav maei dsav maei msei cauli bfai asel/asni/vspi alui rattaatrg tgccgggaag ctagagtaag	nlaIV mspI bsaWI aluI hpaII TTTGTATGG CTTCATTCAG CTCCGGTTCC	sau3AI mbol/ndeII[dam-] fuu4BI dpnI[dam-] sau96I pvuI/bspCI avaII mcrI asuI bsiEI GGTCCTCGA TGTTGTCAG AAGTAAGTTG GCGCCAGTGT CCAGGAGGCT AGCAACAGTC TTCATTCAAC CGGCGTCACA
bsmAl bsal bsal thal thal fnuDII/mvnI bstUI bsh1236I acil ATACGGGGG ACCCAGGTC TATGGGGTC TGGGTGCGAGT CGCGAGGT TATGGCGCTC TGGGTGCGAGCT CACAGGTGCGAGGT TATGGCGCTC TGGGTGCGAGGT CACAGGTGCGAGGT CACAGGTGCAGGT CACAGGTCAGGT	scrFI ncil nspi hpali dsav cauli il/vspi si iT TGCCGGGAL	GG CTTCALTC	ri[dam-] ha ii eae cfr rgrcag AAGTAAGTG ACAGTC TTCATTCAAC
bsmAI bsaI thaI fnuDII/mvnI bstUI ccGGAG ACCCACGCT		CG TTTGGTAT GC AAACCATA	sau3AI mbol/ndeII[dam-] dpnI[dam+] pvul/bspCI mcrI bsiEI cGA TCGTTGTCAG AI sGCT AGCAACAGTC TI
bsmAI bsaI thaI fnuDII/m bstUI bsh1236I aciI aciI	11 bsrI foki T CCATCCAGTC A GGTAGGTCAG	maeIII GTC ACGCTCGT	mnli sau961 p avall m asul b TC GGTCCTCC
fnu4HI bsoFI 11 bsrDI bbvI TGCTGCAATG	mnli acii ATCCGCCT (OGTGGTG?	alui Tagctccttc Atcgaggaag
berl sau961 nlaIV haeIII/pa asuI CTGGCCCCAG	sau961 aval1 asul GCAGAAGTGG TCCTGCAACT TTATCCGCCT CGTCTTCACC AGGACGTGA AATAGGCGGA	cac81 scfl pstl fnu4HI bsoFI bbvI msli bsrDl bsgI sfaNi rGTTGCCATT GCTGCAGGCA TGGTATGG ACAACGGTAA CGACGTCGT AGCATACC	acii AAAAAGCGGT TITITCGCCA
	sal avi asi (CCAGAAGTGG	•	5901 ATGTTGTGCA TACAACACGT
601		A 3 M restable is force altra part and provide the conference and a 10 MeV access to the contract of the contr	and the state of t

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mcrI bsiEI bcgI fnu4HI bsoFI aciI saatagtgt ATGCGGCGAC CGAGTTGCTC	sau3AI mbol/ndeII{ dpnI[dam+] dpnII[dam+] bstYI/xhoII oII alwI[dam-] TTCGG GGCGAAACT CTCAAGGATC	hphi TCACCAGCGT TICTGGGTGA GCAAAAACAG AGTGGTCGCA AAGACCCACT CGTTTTTGTC	sspi TCAAIAITAI IGAAGCAIII AICAGGGIIA AGIIAIAAIA ACIICGIAAA IAGICCCAAI
rsal bsrI scal nlaili sfani maeili hphi csp6i attcrcTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT TAAGAGAATG ACAGTAGGT AGGCATTCTA CGAAAAGACA CTGACCACTC ATGAGTTGGT TCAGTAAGAC TCTTATCACA	PI I/cfol hgiAI/asp II/mvnI bsp1286 II msel bmyl ahaIII/draI GCCACATAGC AGAACTTTAA AAGTGCTCAT	hgial/aspHI bsp1286 bsiHKAI bsiHKAI bsiHKAI bmyI apaLI/snoI mbol/ndeII[dam-] alw441/snoI dpnI[dam+] dpnII[dam+] cccacrcGrG CACCCAACTG ATCTTCAGCA TCTTTTACTT GGGTGAGCAC GTGGGTTGAC TAGAAATGAA	mboli earl/ksp6321 AAGGGAATAA GGGCGACACG GAATGTTGA ATACTCATAC TCTTCCTTTT T TTCCCTTATT CCCGCTGTGC CTTTACAACT TATGAGTATG AGAAGGAAAA A
foki nlaili 6001 ATTCTCTTAC TGTCATGCGT AGGCAN	hgal hinli/acyl ahali/bsaHl hinli/bsaHl hpall scrFI bpall scrFI caull hincll/hindll acil bsh12361 bsh12361 bsh12361 acil bsh2361 acil bsh2361 acil	bsri sau3Al taqi mbof/ndeli[dam-] dpni[dam+] dpni[dam+] acii bstri/xhoii maeli sacii bstri/xhoii maeli AATGGCGACA ACTCTAGGTC AAGCTACATT	acii fnu4HI bsoFi 6301 GAAGGCAAAA TGCCGCAAAA AAGGG

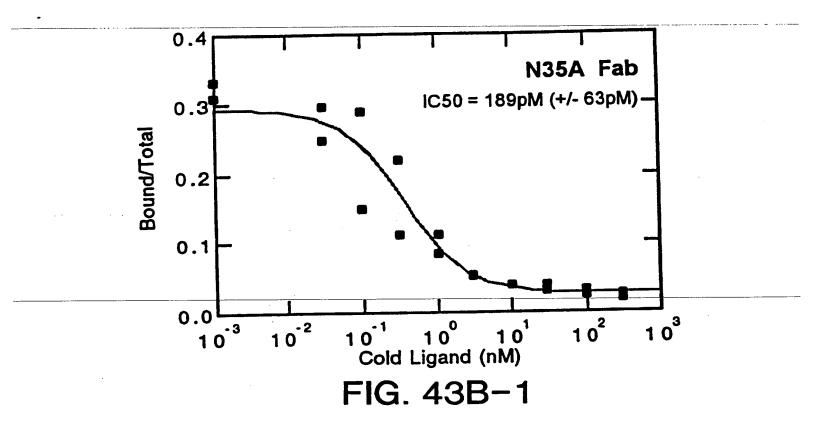
FIG. 41T

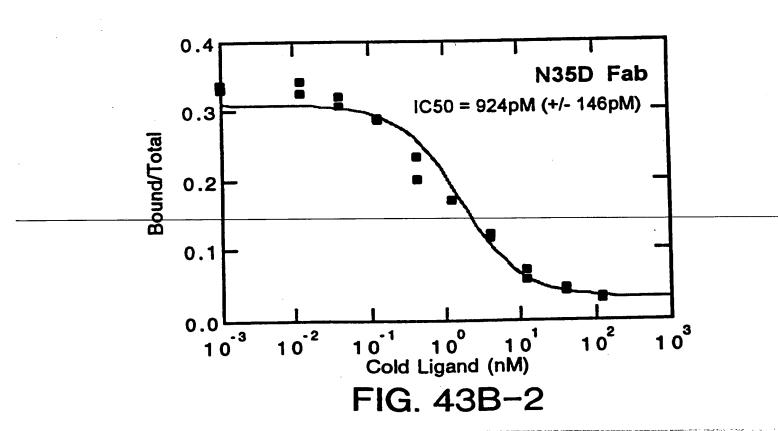
•			
maeII hinl1/acyl ahaI1/baaHI aatII ddeI CCCCGAAAAG TGCCACCTGA CGTCTAAGAA			
	·		
hinPI thai thai fubII/mvnI bstUI acii acii acii acii acii acii acii aci	sau961 haeIII/palI asuI mboII eco0109I/draII mnlI bpuAI bssSI bbsI AACCTATAAA AATAGGCGTA TCACGAGGCC CTTTCGTCTT CAA TTGGATATT TTATCCGCAT AGTGCTCCGG GAAGCAGAA GTT	FIG. 41U	
nlalli rcal bspHI acil bsmAI bsrBI bacccarg AccGGATACA T	nlaili rcai tri bspBi me rcargacari		

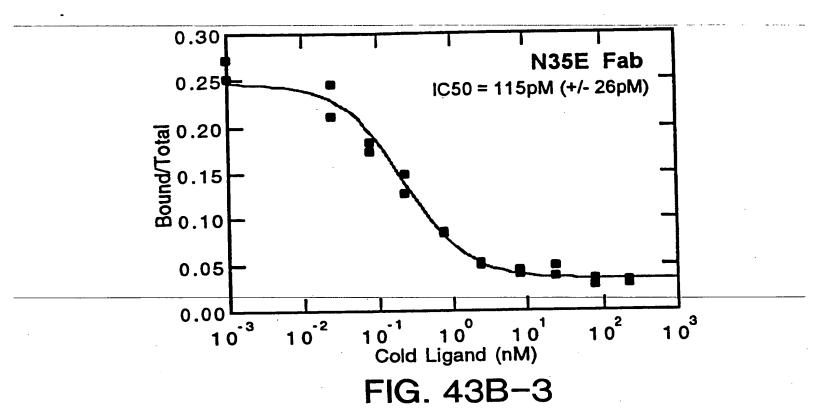
		2 2242 2384 2492 2501 2504 3226 3241 3309 3342 3367 3412 3970 3981 4139 4155 4210 4266 4611 4632 4723 4751 4878 4897 6204 6313 6412 6459		\$ 1386 1906 2054 2075 2126 4845 4935 4981 5238 5759 5859	5 5319 5331 5416 5429 5893	3262 3645 4705 4826 4839	; 2375 2727 3002 3090 3339 3463
	1645 6489 403 823 1093 1963 4449 3867fdam-1	436 351	. 1 18 13 2616 2637 2751 3408 6107 6489	5566 152 320 13 2889	4494 113 71 6214 1385	931 0135 7 1357 1449 1665 1713 1755 1764 2333 4 6166	see thilli 1119 1195 1425 1434 1446 1512 1695 1696 1752 2155 FIG. 41V
>length: 6563	aati(GACGTC): acc51(GGTACC): acc1(GTMKAC):	acii(CCGC):	acyi aflii(ACRYGT): agel(ACCGGT): ahali/bsaHl(GRCGYC): ahalii/dral(TTTAAA):	ahdI/eam1105I(GACNNNNNGTC): 346 aluI(AGCT): 2218 223 5922	alw441/sno1(GTGCAC): alw1[dam-](GGATC): alwN1[dcm-](CAGNNNCTG) apa1(GGGCCC):	apall/snol(GTGCAC): apol(RAATTY): apyl[dcm+](CCWGG): asel/asnl/vspl(ATTAAT) asnl asp700(GAANNNTTC): asp718(GGTACC):	aspI asul(GGNCC):

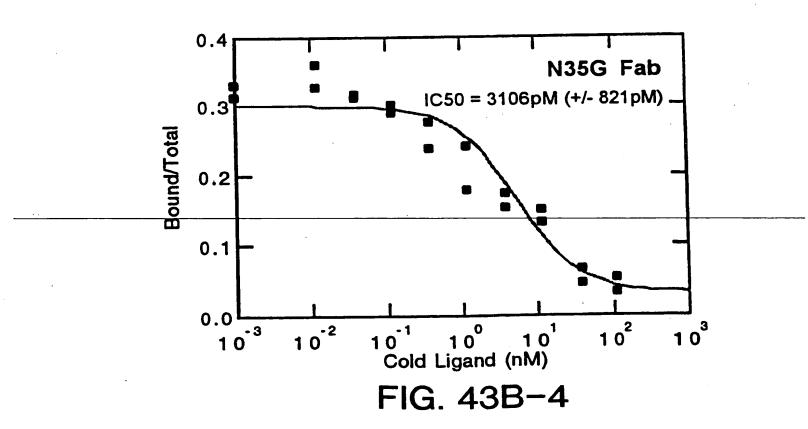
ST TAT TTA CAC 3'	ST TAT TTA CAC 3'		
Stop Template Primer SL.97.2 5' CAT GGT ATA GGT TAA ACT TAT TTA CAC 3'	NNS Randomization Primer SL.97.3 5' CAT GGT ATA GGT NNS ACT TAT TTA CAC 3'	FIG. 42	
Stop Tem SL.97.2	NNS Ran SL.97.3		

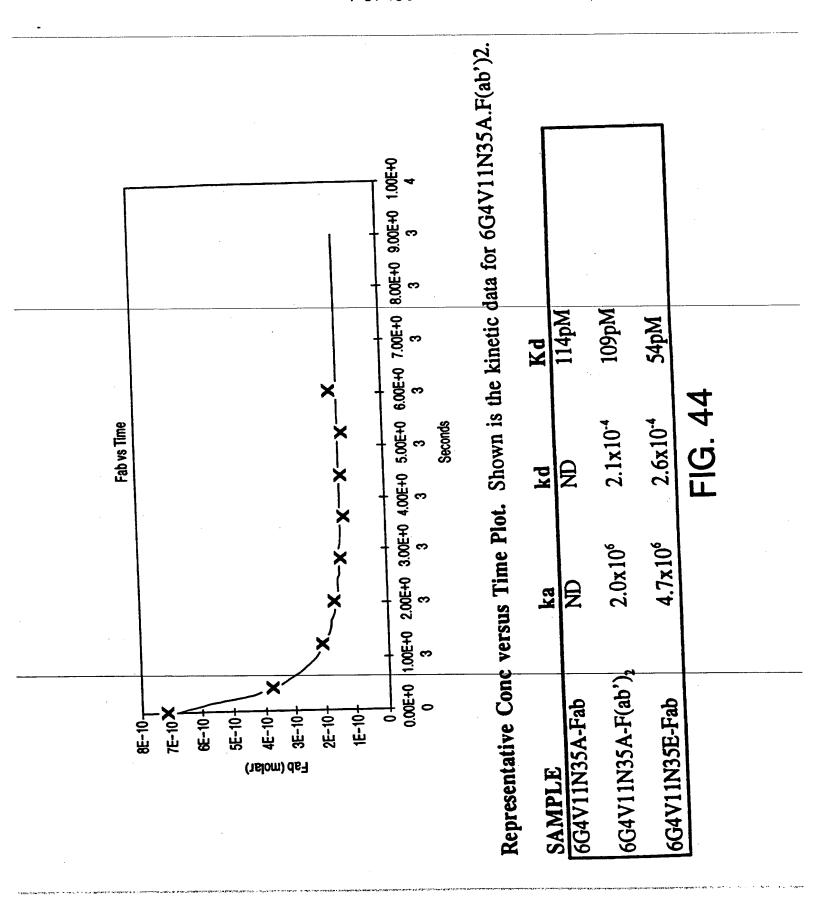
Phage Dienlay			
inder Disting	(NNS Co	don Libro	Display (NNS Codon Library) Sort #3
	Frequency	% Total	IC50 (nM)
Asparagine (wt)		5.6	4.9
Glycine	9	16.6	3.1
Aspartic Acid	3	16.6	3.1
Glutamic Acid	4	22.2	0.1
Alanine	2	5.6	0.2
Lysine	1	5.6	N
Serine	1	1.9	ND
	FIG. 43A	✓	







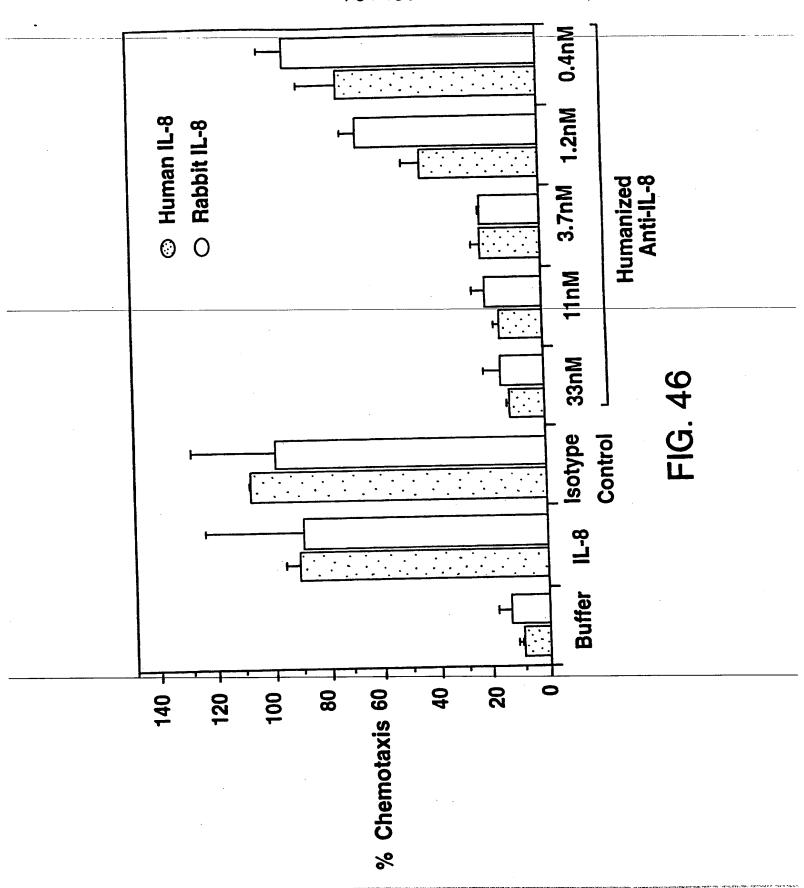




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1	ΣTA	SAA	AAA	GA	AT.	ATC	GCA	TT	TCT	ICT.	160	w y	CI	TAC:	7 J.C.	C^{2}	AAA	AAGA	ATA	ACG	ATG	TTI	'G
	TAC	CTT	TTI	'CT	TA	TAG	CG1	'AA'	AGA	AGA.	ACG	7. N	' A	M I	r L	v	F	S	I	A	${f T}$	N	
								- N (T)	CNC	CC3	-m $-$	c (CG	אכרי	TCC	c 3	rgrc	CGCC	CTC	TGI	'GGG	CGI	\T
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	CG	TAT	GCC	SAC	'I'A	TAC		JIA	CIG	GGI	CAG) 	S	s	L	S	A	S	V	G	D	
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	AP	MG.	KGA T.T	CCA	. 12	4G1	101	T.	P	 G	ĸ	-	A	P	K	L	L	I	Y	K	<u>v</u>	S	
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	T.	rag	CTA	AGA		ACC	105	D D	S	R	F		S	G	s	G	S	G	T	D	F	T	1
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40	1 (	بتكست	rcc	CTG	c o	rga.	ATA	ACT	r ci	TAT	CCC	<b>AGA</b>	GP	/GGC	CAI	AAG	TAC	AGT	GGA	A GO	31G	SW1.	WAC
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60	11	TAC	AGC	CTC	A:	GCA	GC Z	ACCC	T G	ACG	CTG	AGC	: A	AAG	CAG	AC'	r AC	SAGA	MAC	A C	.PAPAPI Teterne	ヘンソン	TAC SATG
17	7 8	Y	s	L	S	9	5	r I		T	L	S	K	A	, D	) :	Υ :	E	K H	l	ν.	٧	•
64	61	GCC	TG	:GA	AG	TC	ACC	CATO	CA G	GGC	CTG	AG	T	CGC	CCG	TC	A CA	AAG!	ハシンと		ZULLA ZULLA	ישבי ישני	GGA CCCT
10	98	Ā	С	E	v	7	r :	H (	2	G	L	S	S	P	V	′ '	T	K :	> 1	-	7.4	*,	~
7:	21	GAC	TG'	TTA.	AG	CTY	GAT	CCT	CT A	CGC	CCGG	AC	G C	ATC	GTC	3GC		MC N	というしょ	יינה ל בינה ל	יע באו יע באו	rca	CGTA GCAT
•		CTC	CAC	TAA	TC	GA	CTA	GGA	GA I	GCC	GCC	TG	CG	TAC	CAC	.cc	Aئ ئ	ICA	100	<b>31</b> .	. GM		GCAT
2	18	E	С	0																			
													_	_									

## FIG. 45

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79/136 s'-ctagtecagtetegegetpecetegtecagecagegegeteactecetttefectgtectetecagettetgectagetactectte-3' S'-TCGAGAAGGAGTAGCCAGAAGCTGCACAGGACAAACGGAGTGAGCCCCCTGG¢TGCACCAGGCCACCGCCAGGCTGCACT Bold indicates nucleotide change destroying Pvull site. N35AH1upr N35AH1lwr

		sau3AI aluI mboI/ndeII[dam-] dpnI[dam+] pvuI/bspCI pvuI/bspCI hinfI taqI[dam-] rmal mcrI pvuII maeI bsiEI nspBII bfaI taqI[dam-] bfaI taqI[dam-] AACTGATTAGGCT GTGGAAAGTC CCAGGCTCCTAGAGGCAATCTTCAG GGGTCCGAGG GGTCCTCTTCAG GGGTCCGAGG GGTCCTTTCAG GGGTCCGAGG GGTCCCGT	sfaNI ppu101 nsil/avaIII nlaIII sphI nspI nspHI cac8I CAGAAGTATG CAAAGCATGC ATCTCAATTA	nlaili tyi coi sai saji caig gcigactaat tittitatt citac cgactgaita aaaaaataa
	DHFR(ID)	bs GTGGAAAGT		s n bsli d acil b cccccc
<del></del>	intron DHFF	CAGTTAGGGT	n+] v cac8I ccccAGCAGG	acii rc cgcccattc ag gcgggtaag
	) and the	GAATGTGTGT CAGTTAGGGT	scrFI mval ecoRII  dsav bstNI apyl[dcm+] dcm+] bsmFI bsmFI cACACCTTC AGGGGTCCGA GG	acil bari erc ceccentre
	nker (pSVI7 ker(LL) int	sau3AI aluI mbol/ndeII[dam-] dpnI[dam+] vul/bspCI dpnII[dam-] taqI[dam-] crI pvulI siEI nspBII qI[dam-] GATC GACAGCTGTG	mval  deav  betni  dcm+)  bemF  Grorcaaag rc	CCCCTAACTC GGGGATTGAG
	3425v11.N35A.choSD the pRK7 cloning linker (pSVI7) and the intron a linearization linker(LL) into the Hpal site	sau3Al alu mbol/ndell[dam+] plel dpnI[dam+] hinfl taql[dam-] mcrl pvul mcrl pvul taql[dam-] taql[dam-]	scrFI mval mval ecoRII dsav dsav bstNI bstNI apyI[dcm bstNI apyI[dcm cagcaAccaG GTGTGGAAAG TCCCCAGGTT GTCGTTGGTC CACACCTTTC AGGGGTCCGA	acil I fokl GCCCATCCG (
	<b>6</b> • • •	sau3AI aluI mbol/ndeIl[dam-] dpnI[dam+] pvul/bspCI pleI dpnII[dam-] hinfI taqI[dam-] rmaI mcrI pvuII maeI bsiEI nspBII bfaI taqI[dam-] bfaI taqI[dam-] cccGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG	el I I CTCAATTAGT GAGITAATCA	benil acii bari acii benil beni acii bari acii  201 GTCAGCAACC ATAGTCCCGC CCCTAACTC GCCCCATTCT  CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAGA
	97 fan/ss.p( one with y adding	STATT STATT	stani ppu101 ns11/av. nla111 sph1 nsp1 nspH1 cac81 aagCatGCAT	acii bemFi NGTCCCGC
	1:27:36 199 //Immbio/af (circular) SSVI backbo	pHI CCCGA( GGGCTC	ppu ppu nsi sphi nspl nspHi cac8i AAGCATG	DE ATAGI TATCI
	> Wed May 7 18:27:36 1997 > /home/ruby/vc/Immbio/afan/ss.p ⁽⁾ > sites: std > length: 8120 (circular) > This has the pSVI backbone with >made from pSVI.WTSD.D by adding	cac81 alu1 sat1 sat1 sat1 hgiJi1 hgiAl/aspHI ecl136II bsp1286 bsiHKAI bmyI taqI trcGAGCTCG CCCGACATTG ATTA	sfani ppuloi nsii/av nlaili sphi nspli nspli cac8i cac8i cac8i cac8i	GTCAGCAACC
	> Wed May 7 > /home/ruby, > sites: std > length: 81 >This has th	स क स	101	201

		017 120	
<b>-</b>	1x1		
	haeIII/pali aluI mcri rmal eagi/xmalii/eclxi maei eaei bfai cfri nhei bsiEi alui hpali CAAAAAGCTA GCTTATCGG	fnu4HI bsoFI bbvI nspBII aciI ATCCCGCTG CCATCATGGT TAGGGGCGAC GCTACTACCA	real cep61 asp700 scal GGACGAGT CAAGTACTTC CCTTGCTCAA GTTCATGAAG
		mnli Agaggattt Tctcctaaaa	III/pall bsrBI acil lcm+] mnli ddei ccrccGcrcA
	rmal maei styi bsaJi bsaJi blni avril[dam-] haelil/pali stul mnll bfaI TTTGGAGGCC TAGGCTTTTG	TAGAGCGATA	hael bacrFI mval ecoRII dsav bstNI apyl [c bsaJI cTACCCTGG
·	mnll mnll bseRI AGAAGTAGTG AGGAGGCTTT TCTTCATCAC TCCTCCGAAA	,	benal baal record of accord refreed to accord refreed of accord a
		maell maelli AG AGTGACGTAA TC TCACTGCATT	GGGAT
	fnu4HI bsoFI bglI sflI haelII/palI mnlI mnlI mnlI aluI mnlI bsaJI acil haelII/palI TATGCAGAGG CGGAGA CTCGATATGC	tf1I hinf1 I II/mvn1 I 2361 GATTCC CCGTGCCAAG	pflMI bslI bamfl aactgcatc ccaaaatatg Ttgacgtagc agcgcacag ggttttatac
	fnu4HI bsoFI sflI haelI/palI ll mnli racil hael ccccc crcccc	aci thai fnub bstu bshi cccc	sfani GCATCG TCGCCG
	fnu4HI bsoFI bsoFI bglI sflI haelII/pal mnlI mnlI mnlI bsaJI acil GAGG CCGAGGCCGC CTC	CGG TGCATTGGAA	Bf ATTG AACTGC IAAC TTGACG
	hae mnli 301 TATGCAGAGG ATACGTCTCC	scrFI noil mspI hpaII dsaV cauII GGCCCTTGCC	taqI 501 TCGACCATTG AGCTGGTAAC
	experience of the second of th	The state of the s	and the second of the second o

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tf11 tru91 hinf1 mse1 dcm+] ddeI mboll tag1 ahaili/dra1 TCTCCATTCC TGAGAAT GGACCTITAA	ធី ថី ប៊	haell/pall hael hael  mval ecoRII dsav tfil dsav bstNI nlaIII bstNI ddel plel ccAGGAAGCC ATGAATCAC CAGGCCACCT TAGACTCTTT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA	
BCLFI mval mval ecoRII dsav bstNI spyl[dcm+] sexAl r TATGGGTAGG AAAACCTGGT TCT	ssti saci hgiJii hgiJii ecll36ii bsp1286 bsiHKAI bmyI. I alui banII ccTCGAGTAA AAGAAC	GITCIGITIA CAAGACAAT	
tfii hinfi hphi alwn[dcm-] A GGTAAACAGA ATCTGGTAGG	aactcaaaga Ttgagtttct	III GGTTTGGATA GTCGGA CCAAACCTAT CAGCCT	•
20571 311 (/ksp6321 TTCAGTGGA	ddel crcagtagag		
ecc mbol early mnll 601 CAAAGAATGA CCACAACCTC 7	tru9I mseI aseI/asnI/vspI 701 AGGACAGAT TATATAGTT TCTGTGTAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA	mspi hpali bsawi 801 ACAACCGGA TTGGCAAGTA TGTTGGCCTT AACCGTTCAT	
9	2	60	

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hgal hlall/acyl ahall/bsaHl scrFI mval mnll ecoRII dsav bstNI ecoNI apyl[dcm+] mnll apyl[dcm+] mnll ramili bsaJI bsll ddel rttatatatc ctctcccaga atacccagc gtcctctc	mnli alui TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT	tru9I mseI aseI/asnI/vspI hattaataca taaccttatG tatcatacac	1
AATATAAAC	sfani mboli Aggaagargc TCCTTCTACG	aluI fnu4HI bsoFI bbvI ACGCAGCTAC	sau96I avalI scrfi mval ecoRII
TGATTTGGG 2	AAAGACTAAC TITCTGAITG	m-] cttcgttaga gaagcaatct	48D
TCCCAGAAAT	I mboli CTACGAGAAG GATGCTCTTC	styl bsaJI sau3AI mbol/ndeII[dam-] dpnI[dam+] dpnII[dam-] alwI[dam-] cTGGCTTTAG ATCCCTTGG CTT GACCGAAATC TAGGGGAACC GAA	FIG. 48[
		ic8I b	
- ] I Taaactti	NI ATCAAGTATA TAGTTCATAT	II bemFI GGGACTTTT	
	scrFI mval ecoRII dsav bstNI apyl[dcm+] sau96I avalI asul mnll sfaNI TCCAGGTCCT CCTTTTTCCG TA	nlal styl ncol dsal baad TATACACCAT	
nlaIII sau3AI mbol/ndeII[dan dpnI[dam+] dpnI[dam+] apcIGGCAAGGA TCATGCAGGA	scrFI mval ecoRII dsaV bstNI apyl[dcm+] sau96I avalI asul mnll sfaNI AGGTCCAGGA GGAAAAAGGC ATCAAGTATA TCCAGGTCCT CCTTTTTCCG TAGTTATA	nlal styl ncol ppu101 dsal nsil/avalli bsaJl ArgCATTTT ATAAGACCAT	
901	1001	1101	

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dsav bstNI apyl[dcm+] mnlI cla1/bsp106 bsaJI crcccAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT GAGGTCCAG GTTGACGTG AGCCAAGATA GCTAACTTAA seq from pRK6G425VH: Cla-AvII1^	scrFI mval fnu4HI ecoRII dsav scrRI bstNi bsoFI bfal apyl[dcm+] bfal acil haell/pall GCTAGTGCAG TCTGGCGTG GCCTGGTGA CGATCACGTC AGACCGCAC CGGACCACGT	scrFI  ncii dsav cauli xmal/pspAi smal smal smal smal sauli bsli scrFi cauli bsli scoRii bsli bsli bsaJi bstNi bsaJi bstNi bsaJi bsli cauli bsli scoNii ly asulbi suuj6i iv asulbi secol091/drali cacccccccccccccccccccccccccccccccccc
+] Caac Git	rmal mael bfal alul A GCTAG	asul asul in a control of control
dsav bstNI apyl[dcm+] mn bsaJI CTCCCAGGTC CAACTGCACC GAGGGTCCAG GTTGACGTGG	-) -1 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3 -3	Bau96 avall ber TATGCACT
bs11 caggtgtcca gtccacaggt	real (/gsul [dcm csp61 scactacatt	pleI hinfi taqi xhoi paeR7i avai maeIII avai maeIII rACTCCTTCT CGAGTCACTA I S F S S H Y Y S F S S H Y Y S F S S H Y
maeili hphi scfi foki GGTGACACTA CCACTGTGAT ATCTATGTA GGTGAAACGG AAAGAGGGT		
CCACTTTGCC	rmal mael bfal TTCTAGTAGC	alui alwni [dcm-] fnu4Hi bsofi bbwi bbwi scretec acttetec
foki Ingataacat Atctattgta	I foki ATCATCCTTT TAGTAGGAAA	
	kI nlalii atgctatgt taccagtaca	hgiJII bep1286 bmyI banII I GGGC TCACTCCGTT CCCC AGTGAGGCAAT
ATACGATTTA	nlalli styl pflMl ncol dsal bsall ccaccarges A	BCTFI BCORI BEAN BEANI BEANI BEANI BOCAGG
1201	1301	1401

thal fnuDII/mvnI bstUI bsh12361 nruI TATCTCGCGA CAACTCCAAA AACACAGCAT ATAGAGCGCT GTTGAGGTTT TTGTGTCGTA	hinli/acyl ahali/bsaHi bsri aatii maelii taqi hphi mboli maeli caarggrac rggrrcrrcg acgrcrgg Grrccacrg accaagaagc rgcagaccc	scrFI mval ecoRII dsav bstNI sauJ  I bspl286 II bsjlKAI bspl286 acil bsaJI TCCAAGAGCA CCTCTGGGGG CACAGGGGC AGTTCTCGT GGACCCC GTGTCGGG S K S T S G G T A A
II[dam-] HaeIII/palI +1 snaBI sau96I] hphi bsaAI CCAATGGT GAAACTACGT ATAATCAAAA GTTCAAGGC CGTTTCACTT GGTTACCA CTTTGATGCA TATTAGTTTT CAAGTGAA N G E T Y N Q K F K G R F T L	cBI mnli ddel drdi cGTGCTGAGG ACACTGCCGT CTATTACTGT GCAAGAGGGG ATTATCGCTA GCACCACTCC TGTGACGGCA GATAATGACA CGTTCTCCCC TAATAGCGAT R A E D T A V Y Y C A R G D Y R Y	sau961 sau961 nlaly hgiJiI bsp1286 ban1  Br ban11  Br ban1  Br ban1
beli sau3al mbol/ndeli(dam-1) dpnii(dam-1) alwi(dam-1) alwi(dam-1) hphi accaaccta atatacacc Trccaatggt accaaccta tataactagg AggTTACCA	Beti bsti bsgi cac81 1601 ACTGCAGAT GAACAGCCTG CGTC TGGACGTCTA CTTGTCGGAC GCAC	esp3I nval bsmBI ecoRII dsav bstNI hphI mlII bsaJI meelII bseRI mt nlalv bstEII bsmAI hae. 1701 TCAAGGAACC CTGGTCACCG TCTCCTCGGC AGTTCCTTGG GACCAGTGGC AGAGGCCG 114 Q G T L V T V S S A

hinpi  hinpi  hasi  kasi  hinli/acyi  hinli/acyi  hasii  h	tfil hinfi maeli CATCTGCAAC GTGAATCACA AGCCCAGCAA GTAGACGTTG CACTTAGTGT TCGGGTCGTT I C N V N H K P S N ahdi/eamll051 sau961 avali	scrfi mval ecoRII dsav bstNI r bsaJI k apyI[dcm ACTCC TGGGC TGAGG ACCCC
maeIII hphI hphI mspI hpaII cfr101/bsrFI bsaMI bsaMI bsaMI ccc gaaccegrea ccGTGTCGTG ageg CTTGCCCACT	fnutHI bsoFI nlaIV rmaI hglCI smoFI banI bboI banI bbvI maeIII bhvI bmyI schGCG TGGTGACTGT GCCTCTAGC TCGTGACT GCGTGATCG TCGTGACT GCGTGATCG TCGAACCTAGC TCGTGAT GCGTGATCG TCGAACCTAGC TCGTGAT GCGTTGGAT GCGTTGGAT GCGTTGGAT GGGTTGGAT GGGTTGGAT GGGTTGGAT GGGTTGGAT GGGTTGGAT GGGTTGGAT GGGTTGGAT GGGTTGGAT GGTTGGAT GGTTGGAT GG T V T V P S S S L G T V T Y	nlaili bapi286 nspi hmyi alwii [ nspii hmyi
ecrFI mvel ecoRII ec NI dsav bstNI bslI apyl[dcm+) fnu4HI bsvI 1801 CTGGCTGCC TGGTCAAGGA CTACT GACCCGACGG ACCAGTTCCT GATGA	ddel plel mnli hinfi ec 811 mnli h bsu361/mstl1/saul ddel 1901 AGTCCTCAGG ACTCTACTCC CTCAGGAGTCC TGAGATGAGG GAGT	benjii benji benji panii panii panii panii panii panii panii caccaagag gacaagaag TTGAGCCCAA GTGTTCCAC CTGTTCTTC AACTCGGTT 214 T K V D K K V E P K

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drdi mnli mboil ddel bpual eco811 bbs1 bsu361/mstII/sauI cGaagacccr GaGGTCAAGT GCTTCTGGGA CTCCAGTTCA E D P E V K F	hphi hgal moli Grestcaccer caccastcec aggastesca v v s v L r v	fnu4HI bsoFI bbvI ccatctccaa agccaaagg g ggtagaggt tcggtttcc g I S K A K G
maeli Acctescea V S H	real csp61 maell beall cacgraccer crecarecea	beal Ascretca Achargeer cecageege arganah Trecagager Tettregga ggereggege Tagetette K v s n K a L P a P I E K T K v S N K A L P A P I E K T
GTGGTGGTGG CACCACCACC	real cep61 AGTACAACAG TCATGTTGTC	LI F CCCAGCCCC A GGGTCGGGG P A P
nlaIII nlaIII mnli nspli ddel msli eco811 mseIII bsu361/mstII/sauI	acil thai thai fuuDiI/mvnI bstUi bsh12361 sacil/sstII hspli kspl dsai bsaJI acil fuu4HI mnlI bsoFI bseRI bsoFI bseRI T R P R E E Q	nnll Acaaagccor TGTTCGGGA R A L
sug6I laIv I [dam-] mnl ddeI ] eco811 sul bsu361 crGGGGACT T P E	acil thai fnubli batui bahl2' acil kapi kapi kapi baaji acil fnu4Hi bsofi GACAAAGCCG CG CTGTTTCGGC GG	
Bau96I nlaIV mspI hpaII scrFI ncil dsaV sau3AI avaII mbol/ndeII[dam-] nlaIII cauII caI dpnI[dam+] c I dpnII[dam+] ec I ATGATCTCCC GGACCCC KG TACTAGAGGG CCTGGGG KG TACTAGAGGG CCTGGGG KG TACTAGAGGG CCTGGGG KG TACTAGAGGG CCTGGGG	ATAATGCCAA TATTACGGTT N A K	real cepti GTACAAGTGC CATGTTCACG Y K C
mep hpa bpa ecrf ncil mbol/ndel nlail caul rcal dpni[dam+ mnli dpnii[dam+ msli bspHi[dam-] ggacaccct argarerece cergreggag racragage	mnli GTGGAGGTGC CACCTCCACG V E V H	ATGGCAAGGA TACCGTTCCT G K E
		GCTGA 1 J
mb II styl earl/ksp6321 bsaJ] CTCTCCCC CAAAACCCAA GAGAAGGGG GTTTGGGTT L F P P K P K	ma real capé bari bae TCAACTGGTA AGTTGACCAT	scrfi mval ecoRII dsav ecoNI bstNI bsrI bsli apyl[dcm+] ccrGCACCAG GACTG GGACGTGGTC CTGAC
2101 C G G G G G G G G G G G G G G G G G G	2201	314

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scrFI mval ecoRII dsav bstNI apyI{dcm+} bspMI c rcaccrccr ggrcanaggc rrcrarcca c rcaccrccr ggrcanaggc rrcrarcca c rcacrcac ggrcanaggc rrcrarcca	plei mlary mboli scfl cac81 recacceac gecretrer recretacac ra cercaccac ccaccacaca accacated ra cercaccac ccaccacaca accacated ra cercaccac ccaccacaca accacate ra cercaccac accacacacacacacacacacacacacacaca
scrFI ncil hpall dsav cauli mal crFI crFI crFI sav sull saJI mbolI saJI matal saJI mbolI	mepi hpali fuuthi bsoFi bsoFi bbvi  GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTG GCCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTG CCGTCGCCGC CTTGTTGATG TTCTGGTGCG GAGGCAC CCGTCGCCCCTC CTTGTTGATG TTCTGGTGCG GAGGCAC  Mboli  mboli bpual bpua
aval saper page 2401 CAGCCCGAG AACCACAGG G	P Q V sal sali cgrccaccac v E W crccacacacacacacacacacacacacacacacacaca

alui fnu4Hi bsofi maelli r TGCAGCTTAT AATGGTTACA	nlaiii alwi(dam-) caaactcatc aatgtatctt atcatgtctg gtttgagtag ttacatagaa tagtacagac	mnli lei acii GA GGCGGAAGA ACCATCTGTG CT CCGCCTTCT TGGTAGACAC
 Pali ACTTGTTTAT TGAACAATA	CAAACTCATCGTTGGTTTGATTGATTACGTTTTGATTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTTACGTACG	rsal csp6I nlaIV kpni hg1CI bani asp718 acc65I ddeI cc ATGGAAGACT
seu961 haelli// II asul nlalli styl ncol dsal (/Pall bsaJl CCATGGCCA	GTGGTTTGTC	CTTGGTTP
alui ndiii AGCTT	rmal mael bsml bfal (TAAAGCAIT TITITCACIG CAITCIAGIT FAITICGIAA AAAAGIGAC GIAAGAICAA	mnli mnli araaceter gaagaggaa tattggaga critciccit
taqi lel sali scfi hincli/hindil lnfi psti lacci bspMi l scr GACCTGCAGI	be T TTTTCACTG	I/pall mnli ga AATAACCTCT CT TTATTGGGA
taqI pleI rmaI salI scfI maeI hincII/hindII sau96I hinfI pstI haeIII/palI bsgI asuI bfaI accI bspMI hi	W. 5-7	fnu4HI hael bsoFI styl bbvI ncol hinpi dsal haelII/palI hhal/cfoI nlaIII /vspI bsaJI ccccrccrc carcccrca AAT
BCIFI  ncil  ncil  mspi  hpali  dsav  bsmAi  bsll cauli  2701 TCCCTGTCTC CGGGTAAATG AG  AGGACAGAG GCCCATTTAC TC  447 S L S P G K O	sfani apoi 2801 aataaagcaa tagcatcaca aaitttcacaa ttatttcgtt atcgtagtgt ttaaagtgtt	sau3AI.  mbol/ndell[dam-] dpnI[dam-] pvul/bspCI mcrI bsiEI tagl[dam-] tru9I clal/bsp106[dam-] bspDI[dam-] mseI bspDI[dam-] mseI bspDI[dam-] asp700 dpnI[dam+] asp700 dpnI[dam-] aseI/asnI/vspI dpnI[dam-] aseI/asnI/vspI cragcragcc crraarraag ccgcaccac
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BOTFI  BVAI  GOORII  GOORII  JABAV  BATIAGE  GAGTTAATTAGE  GAGTTAATCA  GAGTTAA	acil acil foki ccctaattec geceatece ecetaaete gegatteage eggetage	HI I haelil/pali mbli bsexi C TCGGCCTCT GAGCTATTCC AGAAGTAGTG
sfaNI ppu101 nsil/avallI nlallI sph1 sph1 nspHI cac81 carcat	acii bemfi GTCAGCAACC ATAGTCCCGC CAGTCGTTGG TATCAGGGCG	fnud bsof bgll sfil haell mnll ill/pall bsaJl acj ccGAGGCCG
SCIFI  BYAI  CCACAGAGCT  BANI  BANI  BANI  BANI  BANI  BANI  CACTITCAG GGTCCGAGG GTCGTCCGT	sfaNI ppu101 nsil/avallI nlallI aphi nspl nspl nspHI cac81 cac81 cac81 cac41 c	nlaili styi ncol ball daal adii baaji cdccccarg gcrgacraar titititati targcagag gdcgcgcrac gacrgatra aaaaaaraa afacgrcec
3001 GAATGTGTGT CAGTTAGGGT GTG	nlalv scrfi sval ecoRII dsav bstNI apy1[dcm+] bsaJI 3101 TCCCAGGCT CCCAGCAGG CAC AGGGGTCCGA GGGTCGTC GT	berl ecil ac 3201 CGCCCAGTTC CGCCCATTCT CC GGGGGTCAG GCGGGTAAGA GG

SUBSTITUTE SHEET (RULE 26)

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tfil hinfi acil thai fnuDII/mvnI bstUI bsh12361 A CGCGATTCC CCGTGCCAAG AGTCAGGTAA T GCGCTAAGG GGCACGGTTC TCAGTCCATT Ul matched splice donar^	sau3AI mbol/ndeIl[dam-] dpnI[dam+] dpnI[dam-] taqI[dam-] taqI[dam-] clal/bspl06[dam-] bspDI[dam-] dpnI[dam+] dpnI[dam+] dpnI[dam-] aAC CTTTGGATC GATCTACTG ACACTGACAT TTG GAAACCTAG CTAGGATGAC TGTGACTGTA TTG GAAACCTAG TGTGATGAC TGTGATGATGACTAG TAGGATGACTAGATGACTAGATGACTAGATGACTAGATGACTAGATGATGATGATGATGATGATGATGATGATGATGATGA
scrFI  mail mail mail mail mail mail mail mai	fnu4HI bsoFI acil thal sau961 styl fnuDII/mvnl tru9I haelII/pall baJI asuI bsaJI bsaJI asuI bsaJI asuI bsaJI AGGCCACCC CCTTGGCTTC GTTAGAACC GGCTACAATT AATACATAAC TCCGGGTGGG GGAACCGAA CAATCTTCC CCGATGTTAA TTATGTATTG TCCGGGTGGG GGAACCGAA CAATCTTCC App6 promoter  IgG
rmal maeI maeI styl bsaJI bsaJI blu I avrII[dam haeII/palI stuI haeI 13301 AGGAGGCTTT TTTGGAGGC TAGGCT TCCTCCGAAA AAACCTCCGG ATCCGA	acil Bofi Bi capei Bi capei Bi capei Bofi Bofi Bofi Bofi Bofi Bofi Bofi Bof

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nlalli styl pflMl ncol ecoRl dsal apol bsll fokl dam-] bsaJl rrGAATTC CACCATGGGA	BPHI I CCTGTCGCC TCTGTGGGCG GGACACCCGC L S A S V G D	scrfi mval ecoRii dsav bstNi alui apyi{dcm+} AAACCAGGAA AAGCTCCGAA TTTGGTCCTT TTCGAGGCTT K P G K A P K
clal/bspl06 pflM sfaNI fnu4HI ecoRI I bsoFI taqI apoI bbvI bspDI[dam-] rT GGGCTGCATC GATTGAATTC	aluI sstI sstI sacI hgiJII hgiJII hgiJII bsp1286 bsp1286 bsp1286 avaI ssp1 banII ccccGAGCTC GGGGCTCGAG	GTATCAACAG CATAGTTGTC Y Q Q
sau961 avaII maeI maeI avaII maeI bfaI mvaI thaI nheI bstNI fnuDII/mvnI bstNI bsaJI nruI aluI bsaJI crccaGGTCGGG AGCTAGCTT CTCCCAGGTC CAACTGGAA GGGC TTGATGAA caloning linker	bsr bsri ecoRV tth1111/* AGATATCCAG ATGACCCAGT TCTATAGGTC TACTGGGTCA	II I ATTTAC A TAAATC I L
thai fnuDi j mnli baaji wacrecace reser	real bpm1/gsu1[dcm-] sri csp6i ecc. CTG GAGTACATTC AGA1 GAC CTCATGTAAG TCTP	of I I I I I I I I I I I I I I I I I I I
	rmal bpmI bpmI bfil LTTAGTAGCA ACTGCAACTG	rsal ddel alul csp6I hindIII nl rc AAAGCTTAGT ACI AG TTTCGAATCA TG
bell cca caggrerca egg greatera		ecfi psti bsgi sse83871 bspMi bphi bspMi carcaccide aggreaagre gracreda recagreagre I T C R S S Q
CCACTITIC TITITCICCA	nlaiii foki TGGTCATGTA TCATCCTTIT ACCAGTACAT AGTAGGAARA	bphi 88 maelli bsp bstell hphi ATAGGGTCAC CATCACC TATCCCAGTG GTAGTGG
3501 CCACTITITC GGTGAAAAG	nlaili 3601 TGGTCATGTA ACCAGTACAT	3701 ATAG TATG

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fnu4HI bboFI bbvI ifI ifI itI	# 1	naeill/pali nei 11 GGCCAA CCGGIT A K	
fn fn bbs bbstI bbsgI TCTG AGAC	fnu4HI bsoFI bbvI sgcTG	elli el igccaa igccaa igccaa igccaa a K	
fi by be pet: pet: peg caccagnore grogroada	dam-  fnu4    bsoF:   bbvI   ACTGTGGCTG   T V A A T	naelli, hael muli gagaggccaa CTCTCCGGTT E A K	
	sau3al mbol/ndell[dam-] dpnl[dam+] dpnll[dam-] GATCAAACGA ACTGT CTAGTTTGCT TGACA	SGT CA R	
CTCTGACCAT GAGACTGGTA L T I	sau3ai mboi/bdeii[ dpni[dam+] dpnii[dam-] GATCAAACGA CTAGTTTGCT I K R	TTCTATCCCA AAGATAGGGT F Y P R	
TTTCA AAAGT F T	GTGG	xmnI a8p700 IGAATAAC ACTTATTG N N	
T ACGGATTTCA TGCCTAAAGT T D F T	styl styl beajl 51 ccaaggresa GGTTCCACCT	xmnI asp700 GCTGAATAAC CGACTTATTG L N N	
am i j ccc Tr c T T c T T c T T c T T c T T c T T T c T T T T T T T T T T T T T T T T T T T T	real csp61 nlaiv kpni st kpni st kpni st kpni st kpni st kpni bani be asp718 acc651 scc77 GCTA GCTA GCTA GCTA GCTA GCTA GCTA GCTA	Cace 1 CCCGA 1	
mspI hpall bsll bsll sau3AI mbol/ndell[dam-] dpnl[dam-] alw1[dam-] lalv styl/xholl awHI lalv styl/xholl	rsal csp61 nlaiv kpni styl hg1CI bani bsaJI asp718 acc651 GGACAGGGTA CCAAGGTGGA CCTGTCCCAT GGTTCCACCT	cacell TTGTGTGCGT AACACACGGA V C L	
mspl hpall bsll bsll sau3Al mbol/ndell[ dpn1[dam+] alw1[dam-] alw1[dam-] alw1[dam-] alw1[dam-] cGATC CGGTTG		tra > —	
mep hpa bsl bsaW sau3AI mboI/n dpnI[d dpnI[d alwI[da TCTCTGGATC AGAGACCTAG S G S	BI I BREII GCTCACGTTT CGAGTGCAAA L T F	xmn I asp700 TIGGA ACTECTICTE G T A S V G T A S V	
TCTC AGAGI	berBI FI FI CC GCTC	xmnI asp700 sca refector reacce s r a A	
CGCT F F F F F F F F F F F F F F F F F F F	berbl acii bemFi LII GTCCC G(	xeni a sp7 3 sect 7 5 r c 7	
bemFI bpml/gsul[dcm-] 06 pleI -] hinfI TCTGGAGTC CCTTCTCGCT AGACTCAG GGAAGAGGGA S G V P S R F	nla ICAT AGTA H	xmu asignaturasis critageacrix s c	
bsmE bpmI/gsul 106 ple1 m-1 hinfI cTCTGGAGTC GAGACCTCAG S G V	real capé capé capé acai real real real real real real real real	ATGAGCAGTT TACTCGTCAA E Q L	
11 nf1 bsp106 TT CTCT TT CTCT F S		G ATC	
tf11 h1f1 taq1 cla1/bsp106 bsp10f(dam-) hATCGATT CTCT	ACTG	ATCT STAGA	
tfil bpml/gsul claifbapl06 plei bsplidam-] hinfi ccaarcgarr crcrggacre GGTTAGCTAA GAGACTCAG	TTATTACTGT AATAATGACA Y Y C	acii cccccatcic gccctasac p p s d	
		mboli ATCTTC AGAAG	
TACAAAGTAT ATGTTTCATA Y K V S	TTCG(	ooli Mai mboli CITCATCTTC GAAGTAGAAG F I F	
	mboll bpual bbsl crrc re	mboll bpual bbsl GT CTJ ACA GAP	
ACTACTGATT TGATGACTAA L L I	mboli bpual bbsi cagccagaag Actrogcaag grogicito igaagceiig aataaigaca	CATCI STAG	
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3801 51	3901	4001	

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fnu4HI ddel bsoFI scfI mnll bbvI GCAGGACAGCA CCTACAGCCT CAGCACCC GGTCTGTCG TTCTGTCGT GGATGTCGGA GTCGTCGTG	saci hgiJii hgiAi/aspHi ecil36ii bspl286 bsiHKAi	ddel cac81 haeIII/pall sau961 alu1 asu1 banII ecool091/drall alwN1[dcm-] alwN1[dcm-] cAGGGCTGA GCTCGCCGG CACAAAGAG TTCAACAGGG GTCCCGGACT CGAGCGGCA GTGTTTCTCG AAGTTGTCCC GTCCCGCACT CGAGCGGCA GTGTTTCTCG AAGTTGTCCC GTCCCGCACT CGAGCGGCA GTGTTTCTCG AAGTTGTCCC GTCCCGCACT CGAGCGGGCA GTGTTTCTCG AAGTTGTCCC GTCCCGCACT CGAGCGGCA GTGTTTCTCG AAGTTGTCCC GT CCCGACT CGAGCGGCA GTGTTTCTCG AAGTTGTCCC GT CCCGCACT CGAGCGGCA GTGTTTCTCG AAGTTGTCCC	finuthi beofi beofi beofi broi recagetrat antalageaa tracerence antitegeret recagetrat traceret trateget trategere aggregata traceret arcerage trategere
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maeiii TGTCACAGA ACAGTGTCT V T E		hphI maeIII AGTCACCCAT TCAGTGGGTA V T H	alul fuutil bsoFI maeIII bbvI maeIIII bbvI maeIIII bFI TGCAGCTTAT AATGGTTACA TGAACAATAT ACTCGAATA TTACCAATGT
		cac81 ACGCTGCGA TGCGGACGCT A C E	find find filbeofi beofi beofi cagaran
scrfi mvai mvai ecoRII dsav bstNI apyl (dcm- maeIII bsaJI ATCGGTAC TCCCAGGAGA TAGCCCATG AGGGTCCTCT		acol CACAAAGTCT GTGTTTCAGA H K V Y	
mnlI ball rececencea A L Q		CTACGAGAAA GAIGCICTI Y E K	sau961 acii haeili/pali fuu4Hi asui bsoFi nlaili alui haeili/pali ndili bgli ncoi cfri bsaJi cfri bsaJi rcGACCGC GGTACCGGT TGA
AAGGTGGATA TTCCACCTAT K V D N		espi pull021 gcaaagc? cgtttcgi	acii fnu4H bsoFi alui hselli hindili bgli tru9I eael msel cfri msel cfri cccrcacat rcgaccecc
real cap61 AGTACAGTGG TCATGTCACC			
4101		4201	4301

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eII[dam-] am-] cI ci l l tru9i fnu4Hi hael neel bBoFi styl neal bbvi ncol xmni hinpi dsai haelII/pali asp700 hhal/cfoi nlaili GAATTAATTC GGGGAGGAC CATGGCTGA cTTAATTAAG CCGCGTCGTG GTACCGACT	scrFI mval ecoRII dsav bstNI apyl [dcm+] bsaJI bsaJI bsaJI cAGTTAGGGT GTGGAAAGTC CCCAGGCTCC GTCAATCCCA CACCTTTCAG GGGTCCGAGG sfaNI nballI nballI sphl	nspi nspki cac81 cac81 cccagcage cacaagtate caagcatec gegregree grettcatae grtectaeg
sau3AI mboI/nd dpnI[da dpnI[da dpnI][dam-I bs1EI taq1[dam-I bspD][dam-I bspD][dam-I bspD][dam-I bspD][dam-I bspDI[dam-I dpnI[dam-I and [dam-I dpnI[dam-I cangener and tagner and	sal sp61 sp61 sp10 sp10 no muli pvuli p718 muli pvuli c651 ddel acii nspBili TACCTTCTGA GGCGGAAGA ACCAGCTGTG ATGGAAGACT CCGCCTTTCT TGGTCGACC CTTACACACA  scrFi mval ecoRii d8aV	petni apyl[dcm+] BexAl  CTCAATTAGT CACCAACCAG GTGTGGAA  GAGTTAATCA GTGTTGGTC CACACCTT  FIG. 48F
rmal mael bsml bfal drGTTTGTC AAAAAGTGAC GTAAGATCAA CACCAAACAG	### TATTGAGA CTTCTCTT GAACCAATCC  #### ##############################	sphi nspi nspi cac81 4601 CCAGCAGGCA GAAGTATGCA GGTCGTCCGT CTTCATACGT TTCGTACGTA

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nlaIII styl ncol ncol bsll dsal c CGCCCATTCT CCGCCCATG GCGGGGTAC CGACTGATTA	rmai maei styi bsaji blai avrII[dam-] haeIII/pali stul maeI maeIII mnll bfaI TTTGGAGGCC TAGGCTTTTG CAAAAAGCTG AAACCTCCGG ATCCGAAAAC start PUC118^	scrfi mvai ecoRII bstvi maeli maelii bsaji cGTTTTACAA CGTCGTGACT GGGAAAACCC GCAAAATGTT GCAGCACTGA CCCTTTTGG
acil acil foki ccctaactcc cccctaactc GGCCAGTI GGGATTGAGG CGGGGTCAAGT	fnu4HI bsoFI bglI sfiI haeIII/palI ddeI mnlI mnlI mnlI lbsaJI mcli haeIII/palI bsaJI acit haeIII/palI GG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC CC GGCCCGC GAGCCGAGA CTCGATAAGG TCCTCCGAAA	hhal/cfol hhal/cfol funDII/mvnI bstUl hhal/cfol cac8I tru9I pstI ascI ahaIII/draI cac8I tru9I pstI ascI ahaIII/draI fru9I bsh1236I mseI bsgI maeIII mseI bssHII swaI sse8387I aluI bsrI ATTAAGGCG GCATTTAAA TCCTGCAGGT AACAGCTTG CACTGGCCGT TAATTCCGCG CGCTAAATTT AGGACGTCCA TTGTCGAAC GTGACCGCAA  TAATTAAGGCG CGCTAAATTT AGGACGTCCA TTGTCGAAC GTGACCGCCAATINAA ISTE
acii bemfi 4701 ATCTCAATTA GTCAGCAACC ATAGTCCCGC	heeli mnli be 4801 TTTTTTATT TATGCAGAG CC AAAAAATAA ATACGTCTCC GG	fnu4HI haeIII/pall hha mcri eagl/xmaIII/eclXI thal eagl/xmaIII/eclXI thal eagl/xmaIII/eclXI thal eagl/xmaIII/eclXI thal eagl/xmaIII/eclXI thal eagl/xmaIII/eclXI thal fnutI barBI bsoFI xhoI fnutHI tru9I paeR7I bsiEI pacI avaI bsoFI mseI tru9I bsh; mnII aciI aciI mseI bsh; mnII aciI aciI mseI bsh; mnII aciI aciI mseI bsh; avaI bsoFI cccGcCGCTA AITAAGCCC AATGGAGCTC GCCGCGAAT TAATACCGCG AInearization linker inserted

nlaIV hgici taqi bani mnli A GTGCTTACG GCACCTCGAC	maeli plei tru9i plei drdi hinfi maeli msei hinfi TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA	tru91 msel tru91 haeIII/pal1 GCCGATTTCG GCCTATTGGT TAAAAATGA GCTGATTTAA CGGCTAAAGC CGGATAACCA ATTTTTACT CGACTAAATT	acil fnu4HI bsoFi tru9I sfaNI msel acil CT GCTCTGATGC CGCATAGTTA AGCCAACTCC	sfaNI mspI hpaII scrFI nciI dsaV fokI cauII aciI cc cGAACAGACG AGGCCGTAG GCGAATGTCT	
16 nlaIV CCCTTTAGG TCCGATTTA GGGAAATCCC AAGGCTAAAT	maell ple drdi hir Atagacegtt Titceccctt Tgacetigga Tatcieccaa aaagcegeaa Actecaacct	AAGGGATTTT GCCGATTTCG TTCCCTAAAA CGGCTAAAGC	hgial/aspHI bsp1286 bsiHKal bmyl ddel apaLI/snol rsal alw441/snol csp61 GTGCACTCTC AGTACAATCT	hinPI hhal/cfol thal thal fhubli/mvnl bstUl nspBII bsh12361 acii acii hgal drdi cccccaaca cccctGacGc GccctGacGc	48S
nlalv hgijil bspi2e bmyi banii ATCGGGGCT	TG ATAGACGGTT	TTTGATTTAT AAACTAAATA	I DEI TTA CAATTTTATG AAT GTTAAAATAC		FIG. 48S
/bsrFI aluI ctttcccct caactctaa gaaagggca gttcgagaff	maeli haelii/pali raili sau96i saali asul R CGTAGTGGGC CATCGCCTG	bsli bsli aval accctatctc gggctattct tgggatagag cccgataaga	tru91 maell psp14061 msel sspl msel sspl msel TTAACAAAATA TTAACGITTA	hinpl fnu4HI baoFI nlaIII hhal/cfol aspl bbvl TCATGGCTGC GCCCGACAC	
mspl hpall nael cfrlol maell cac8l TTTCTCGCCA CGTTCGCCGG	me dra hphi bas TTGATTTGG TGATGGTTCA	bsrI CCAAACTGGA ACAACACTCA GGTTTGACCT TGTTGTGAGT	thal funDII/myn tru9I apol tr msel bstUI ms apol bsh1236I CAAAATTTA ACGCGAATTT GTTTTAAAT TGCGCTTAAA	maelli maeli bari baal tthilii, GCTATCGCTA CGTGACTGGG	
5301	5401	5501	5601	5701	

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mnli haelil/pall haelil/pall sau961 bpuAl asuI bsSI bbsI ecol091/drall agrattcttg aagacgaaag ggcctcgtga	nlalv acii thai thai fuuDil/mvni bstUi bsh1236i hinPi hhal/cfoi AAATGTGGGG CCTTGGGGAT AAACAATAA	mboli earl/ksp6321 msli aaaaggaaga gtatgagtat tcaacatttc tttccttct catactcata agttgtaaag	bsp1286 sau3AI bsp1286 mbol/ndeII[dam-] dpnI[dam+] bmyI dpnII[dam-] eco57I apaLI/snoI sfaNI mbolI[dam-] alw441/sn AAGTAAAAGA TGCTGAAGT CAGTTGGGTG TTCATTTTCT ACGACTTCTA GTCAACCCAC	
thal fnuDII/mvnl betUI bsh1236I hinPI hhal/cfoI thal mnlI fnuDII/mvnI bstUI CGCGCGAGC A	CTTTTCGGGG A	sspl ATAATATTGA ? TATTATAACT	hphi Acgctggtga Tgcgaccact	
hphi hphi TTTCACCGAAA AAAGTGGCAG TAGTGGCTTT	hinll/acyl ehall/bsaHl aatll ddel maell TTCTTAGACG TCAGGTGGCA	TAACCCTGAT AAATGCTTCA ATTGGGACTA TTTACGAAGT	CTGTTTTGC GACANAACG	
I mnli TGTCAGAGGT ACAGTCTCCA	TAATAATGGT ATTATTACCA	rcel bepHI 31 bemAI 11 nleIII 27 CATGAGACAA 3A GTACTCTGTT	TTTGCCTTC	
nspi nspi fnutti bsofi bbvi alui nlaii GAGCTGCATG	nlaili 191 real 11 bspHI AATGTCATGA	berBi BerBi Beil TGTATCCGCT ACATAGGCGA	fnu4HI bsofI acii TTTTGGGGA	w
BCTFI DCII DCII MBPI HPRII BBRI DSMBI BRMBI ALUI BBMAI ALUI BBLI CRUII CAAGCTGTGA CCGTCTCGG G	tru mse TACGCCTATT TTTATAGGTT ATGCGGATAA AAATATCCAA	TTTCTAAATA CATTCAAATA AAAGATTTAT GTAAGTTTAT	6101 CGTGTCGCCC TTATTCCCTT TT GCACAGCGGG AATAAGGGAA AA	
5801	5901	6001	61	

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	100		1
hgial/aspHI bspl286 tru9I bsiHKAI mseI bmyI ahaIII/draI ATGAGCACTT TTAAAGTTCT	rsal csp61 bsr1 scal hph1 maeIII TGGTTGAGTA CTCACCAGTC	sau3Al  aeI frI frI dpnI{dam+} dpnI{dam+} dpnII{dam-}  u4HI pvuI/bspCI mcrI ll bs1EI CCG CTACTTACTT CTGACAACGA	nlaili sau3Al maelli mbol/ndell[dam-] dpnl[dam+] dpnl[dam+] dpnl[dam-] dpnl[dam-] dpnl[dam-] dpnl[dam-] bawi T GGGGATCAT GTAACTCGCC TTGATGGTG GGAACCGGAG CTGAATGAAG A CCCCTAGTA CATTGAGCG AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT  FIG. 48U
	AGAATGACT TCTTACTGA	hael eael cfrl fnu4HI bsoFI acll acll acll	mspI nlaIV I[dam-] alu hpaII -] bsaWI GGAACCGGAG C
dam-] psp14061 xmn1 asp700 mbol1 AGTTTTCGCC CCGAAGAAGGTTAC	acil  mcri fnu4Hi  bcgi baiEi bsoFi  GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT TGGTTGAGTA  CTCGTTGAGC CAGCGGCGTA TGTGATAAGA GTCTTACTGA ACCAACTCAT		nlaili sau3Al maeili mbol/ndeil[dam-] dpni[dam+] dpni[dam-] dpni[dam-] dpnii[dam-] dpnii[dam-] dpnii[dam-] dpnii[dam-] dpnii[dam-] dpnii[dam-] bsaWi GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG CTGAATGAAG CCCCCTAGTA CATTGAGCGG AACTAGCAAC CCTTGGCCTC GACTTACTTC  FIG. 48U
	acii mcri fnu4Hi baiEi baoFi cc Grcccccar	fnu4HI baoFI bbvI mslI nlaIII TATGCAGTGC TGCCATAACC ATGAGTGATA	nlaili sau3Ai maeili mboi/ndeil[dam-] dpni[dam+] alwi[dam-] sigarcar graactcgcc cccragra carrgagcgg
nspBII sau3AI II[dam-] mbol/ndeII[dam-] +] dpnI[dam+] II dpnI[dam-] m-] alwI[dam-] AcaccGTAA GATCCTTGAG AGTTT TGTCGCCATT CTAGGAACTC TCAAA			nlaili sau3Ai ma mboi/ndei dpni[dam+ dpnii[dam+] II GGGGATCAT GT IA CCCCCTAGTA CA
	scrFI ncii mspi hpali dsav hinli/acyi hgal cauli ahali/bsaHI CCCGTGATGA CGCGGGGAA	nlaiii Gatgaca gtaagagaat Gtactgt cattctctta	n 1 TGCACAACA ACGTGTTGT
sau3AI mboL/nde dpnI[dan bstXI/xhc bstSI maeIII taqI alwI[dan cacGaGTGGG TTACATCGAA CTGGATCTCA GTGCTCACCC AATGTAGCTT GACCTAGAGT	acil thai thai fuuDil/mvni bstui bsh1236i hinPi hhal/cfoi GCTATGTGGC GCGCTATTAT CCCGT	INI fokI ATCTTACGGA TGG TAGAATGCCT ACC	alui Bargarcta Cttcctcgat
bsesi 6201 CACGAGT GTGCTCA	6301 GCTATGT	sfe 6401 ACAGAAAAGC TGTCTTTTCG	sau96 avaII asuI mnlI 6501 TCGGAGGACC
	SHRSTITU	FE CUEET (BIN E AC	•

		1017130		dam- )
tru91 mseI aseI/asnI/vspI AAAATTAATA	mspl hpall cfrl01/bsrFl IV hphl bsmAl sul[dcm-] bsal GCCGGT GAGCGTGGGT	I TG AACGAAATAG AC TTGCTTTATC	tru9I msel ahaIII/draI mseI ATTTAAAACT TCATTTTTAA	sau3AI mbol/ndeII[dam-] dpnI[dam+] dpnII[dam-] CC GTAGAAAGA GG CATCTTTCT
mspi hpali alul scrfi rmal ncil mael dsav bfal cauli cTTACTCTAG CTTCCCGGCA	mspI hpall cfr101/bsrFI nlaIV hphI bpmI/gsul[dcm-] cTGATAAATC TGGAGCCGGT GAGCGTGGGT	pleI hinfI m1105I fokI gagrcaggca acrarggarg crcagrccgr rgaraccrac		hgal ddel TCCACTGAGC GTCAGACCCC AGGTGACTCG CAGTCTGGGG
 bbri 191 NAC TGGCGAACTA CIT		ahdI/ee ACACGACGGG TGTGCTGCCC	CTCATATATA GAGTATATAT	GAGTTTTCGT
ifol ispl tru mse AACTATTZ	bgli cac81 sau961 cac81 haelil/pali avali hinPi asul mspl asul hhal/cfol hpali ACTTGCAGGA CCACTTTGGCTGC TGGTTTATTG	111 CTCCCGTATC GTAGTTATCT GAGGGCATAG CATCAATAGA	IGTCAG ACCAAGTTTA	maeII tru9I maeI caaaat cccttaacgt
hinPl hhal/o msti avill/f DI psp1406I AATGCCACA ACGTGCGCA	bgli sau961 hae111, t hinpi asui hhal/cfoi ccactrctGC GCTGGGCCT	mr Taagcc	ddel nlaIV mbol/ndeII{dam-} dpnI{dam+} hgiCI mseI maeIII dpnII{dam-} banI mn]I mseI maeIII ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC	dam-) nlaIII rcaI bspHI GATAATCTCA TGACCAAAAT CTATTAGAGT ACTGGTTTTA
fnu4HI bsoFI cac81 bsrDI I bbvI GCCAGCAGC AA	sau96I avaII asuI GTTGCAGGA CC	haeIII/palI sau96I nlaIV i asuI rGGGCCAG ATGG	tru91 scrcacrcar rad	sau3AI hi mbol/ndeli[dam- i[dam-] ] dpni[dam-] i alwi[dam-] bsti/xhoii mboil[dam-] rGAA GATCCTTTT GATA
fnu4HI bsoFI mall cac81 bsrDI maeIII sfaNI bbvI ps	foki acii avali beri avali beri muli carcigates Accecata Actrocaca CCAC		ddel nlalv mbol/ndell[dam-] dpnl[dam+] hglCl dpnIl[dam-] banl mnll GATCGCT GAGATAGGTG CCTC	ohi [1[dam 1-] [1] be mbol srgaa
me 6601 CGACGAGCGI GCTGCTCGCA	fokI bsri n 6701 Gactggatge CTGACCTACC	acii thai fnuDii/mvni bstui bsh1236i bs 6801 CTCGCGGTAT CA GAGCGCCATA GT	ddel sau3Al mbol/ndell{ dpnl{dam+} dpnl[dam-} dpnll{dam-} e901 ACAGATCGCT GAG	rmal mael sau3AI hg mbol/ndel dpnI[dam-dpnI[dam-tru9I bstxI/xho] msel alwI[dam-1 ahaIII/draI bfaI 7001 TTTAAAAGGA TCTAGG
 99		<b>9</b>	· · · · · · · · · · · · · · · · · · ·	<b>.</b>

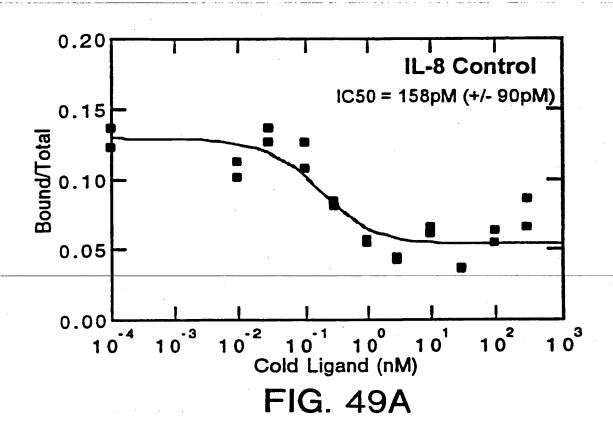
·			
sau3AI mboI/ndeII[dam-] dpnI[dam+] dpnII[dam-] alwI[dam-] mspI hpaII aluI GTTTGTTGC CGGATCAAGA	pali A CTTCAAGAAC I GAAGTTCTTG	scrFI ncil nspl hpall dsaV pleI caull hinfi cGTGTCTTAC CGGGTTGGAC TCAAGACGAT	scfI T ACTACAGCG A TGGATGTCGC
	haeIII/palI haeI TAGGCCACCA CT	scrFi ncii mspi hpali dsav cauli hinf c cccTTGGAC	ddel Gaactgagat G Cttgactcta
acii nspbii ACCAGGGTG	bsl1 TAGCCGTAGT ATCGGCATCA		GACCTACACC
aci I AACCACGCT TTGGTGGCGA	rmal mael bfal ccrrcragre GGAAGATCAC	GGCGATAAGT	hgial/aspHI bsp1286 bsihkal bmyI apaLI/snoI alw44I/snoI alw46CGCACA CAGCCCAGCT TGGAGCGAAC AAGCACGTGT GTGGGTCGA ACCTCGCTTG
Caacaaaa	CAAATACTGT GTTTATGACA	fnu4HI bsoFI bbvI fnu4HI [dcm-] bsoFI bbvI GC TGCTGCCAGT	hgial/asphi bsp1286 bsihkai bmyi apali/snoi alw441/snoi alwi Trccrccaca cacccacct AAGCACGTGT GTCGGTCGA
cac81 fnu4HI bsoFI GCTGCTTG	hinpi hhal/cfol GCGCAGATAC CGCGTCTATG	fn bs fnu4HI alwNI (dcm-) bsrI bsoFI maeIII bbvI GT TACCAGTGGC TGC	hgial/aspHI bsp1286 bsiHKAI bmyI apaLI/snoI alw44I/snoI TTCGTGCACA CAG AAGCACGTGT GTC
thai fnuDil/mv batui bah1236I hinPi hhal/cfoi TGCGCGTAAT	eco57I CTTCAGCAGA GAAGTCGTCT	fnu4HI bsoFI bbvI fnu4HI alwNI[dcm-] bsrI bsoFI maeIII bbvI bsrI cTAATCCTGT TACCAGTGGC TGCTGCCAGT GCGATAAGT	GAACGGGGG
AI  IndeII[dam-]  I(dam-)  I(dam-)  I(dam-)  CCTTTTTC  GGAAAAAAAG	bsrI maeIII AGGTAACTGG TCCATTGACC	mnli ccrcgcrcrg ggagcgagac	acil 14HI 14HI 17 mcI bsiti cfol cGCTCGGCT CCCCGA
sau3AI mboII[dam-] sau3AI mboI/n mboi/ndeII[dam-] dpnI[dam+] dpnI[d dpnI[dam-] dpnII[dam-] stYI/xhoII alwI[d lwI[dam-] bstYI/x GATC TTCTTGAGAT CC	CTTTTTCCGA GAAAAAĞGCT	acii c cectacata c cccatetat	ns fnu bsc bbv hinPI TAAGGGGCA ATTCCGCGTA
sau3 mboll[dam-] sau3Al mbol mbol/ndell[dam-] dpnl[dam+] dpnl dpnll[dam-] dpnl bstYl/xholl alw] alw1[dam-] bstYl TCAAAGGATC TTCTTGAGAT	GCTACCAACT CTTTTTCCGA CGATGGTTGA GAAAAAGGCT	sofi schi Totgrageac egectacata Agacategig eeggaigtat	mepi hpali bsawi maelli AGTTACCGA TCAATGGCCT
71017	7201 (	7301	7401

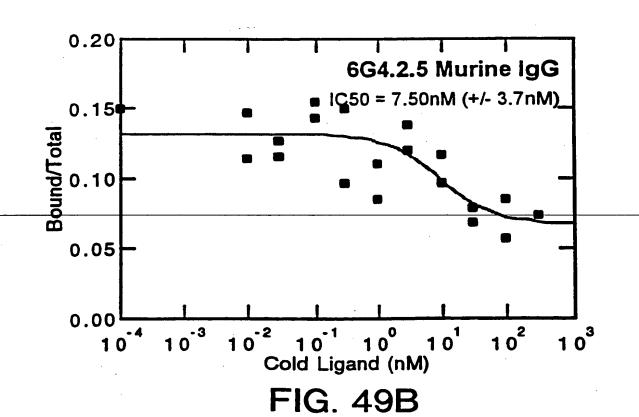
•				
SCIFI mvai mvai ecoRII dsav bssSI bstNI hinpi mnli bsaJI hhal/cfoi alui apyi{dcm+} ccitgrcctc rcgccrgctc ccrcGAAGGT	nlaIV sfaNI GATGCTCGTC AGGGGGGGG AGCCTATGGA CTACGAGCAG TCCCCCGCC TCGGATACCT	tfii hinfi TGCGTTATCC CCTGATTCTG TGGATAACCG ACGCAATAGG GGACTAAGAC ACCTATTGGC	sapi hinpi mboli hhal/cfol eari/ksp6321 mnli acii haeli GAGGAAGCGG AAGAGCGCC AATACGCAAA CTCCTTCGCC TTCTCGCGGG TTATGCGTTT	
mspI hpall fnu4HI ball bsoFI acii bsaWi acii cqcTTCCCGA AGGGAGAAG GCGACAGGTC	taqi mnli drdi hgal rraragrect gregegitic eceaectera aetreagese egatititist aarareagsa eageecaaag eggigeagae igaaetegea getaaaaaga	pall haeIII/pall   scrf!   mval bsl!   ecoRII   nla!!    dsav   haeIII/pall nsp!   apyl[dcm+] hael   afli!    nla!v hael   afli!    TITTTACGGT TCCTGGCCTT TTGCTGCCT TTTGTTCTTTCC	fnu4HI bsoFI bbvI bbvI bsrBI fnu4HI bsrBI fnu4HI acil bsoFI acil bsoFI bstEI hhal/cfoI acil bsoFI bstEI hhal/cfoI ACTATGGCG AGCGCGTCG CGACGACG ACTATGGCG AGCGCGTCG CATCATCA	
hinPI hhal/cfol hael/ 7501 TGAGCATTGA GAAAGCGCCA CG ACTCGTAACT CTTCGCGGT GG	scrFI mval mval ecoRII dsav bstNI apyI[dcm+] ccccTTGC GGACCATAGA AA	haeIII/palI fnu4HI bsoFI acil thaI bslI fnuDII/mvnI bstUI 7701 AAAACGCCAG CAACGCGGC TITTF	barb acii alui acii 7801 TATTACCGCC TTTGAGTGAG CTGATACCGC ATAATGGCGG AAACTCACTC GACTATGGCG	

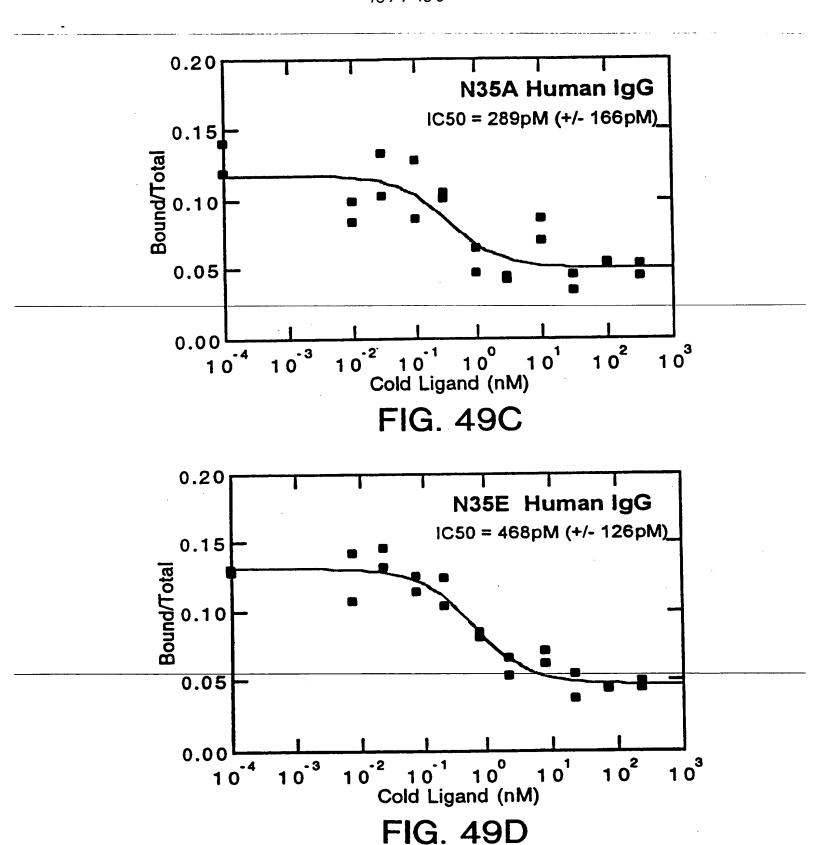
thai betui alui betui alui betui bet	mval ecoRII dsay  hgicl apyl[dcm+] mspI mnll ban1 bsad1  acticactor tragecacc cagcottrate represent treascace transcare transcare criticical representation and testing the contraction and testing transcared transcared containing the contraction and testing transcared transca
cac81 TGGAAAGGG	aci. bsrBI GAATTGTGAG C
beri GTTTCCCGAC TG	ATGTTGTGG TACAACACAC
I GGCACGACAG CCGTGTG	mspI hpali TccgcTcGT AggccGAGCA
cac8I  (pall alul  fil asel/asnl/vspl infi msel nspBII  ATTCAT TAATCCAGCT G	ACTTTATGCT TGAATACGA
nI III/Pali tri ase hinfi ms	scrFI mval ecdRII dsdv bstNI apyI[dcm+] sadI
thai fuuDII/mvnI bstUI bsh12361 hinPI hhaI/cfoI thai fnuDII/mvnI bstUI haeIII, bsh12361 bsh12361 cccccccTTG GCCG GGCGCCAAC CGGC	scrFI mval eccRII dsav nlaIV bstNI bgiCI apyI[dcm+] banI bsaJI TTAGGCACCC CAGGCTTT
mnll acil ccccrcrcc gcccrcrcc	mn1I ACCTCACTCA TGGAGTGAGT
06 106 106	10 8 8 11 F 26)

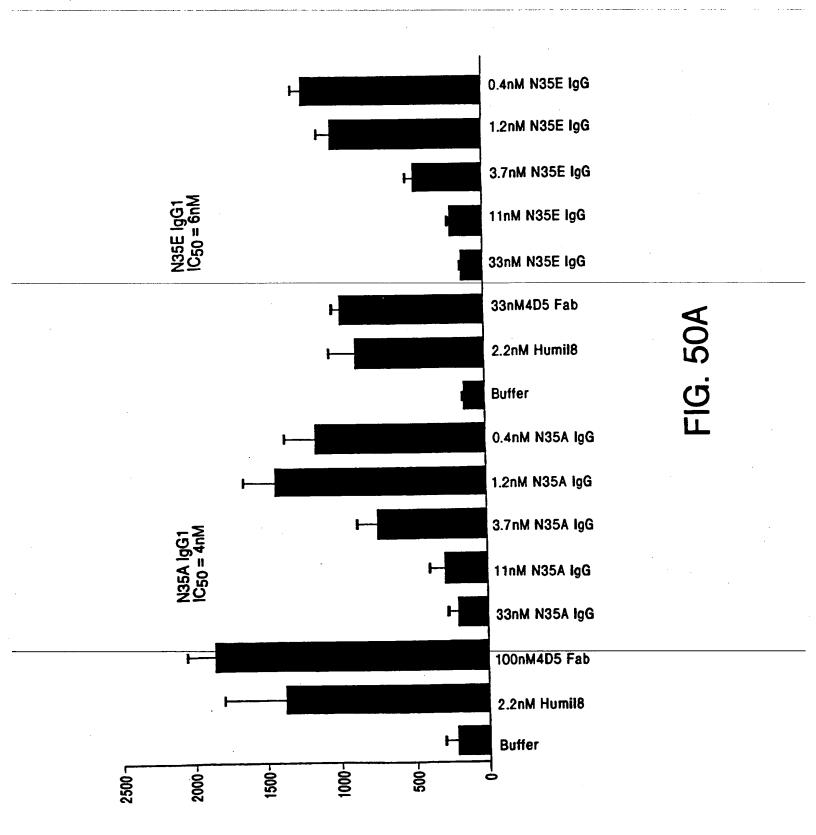
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		271 317 422 454 485 574 1385 1795 1871 2248 2250 2758 2 3210 3221 3267 3372 3404 3449 3686 3949 4021 4318 4542 4781 4827 4910 4914 5070 5127 5153 5166 5203 5217 5220 5751 5790 5979 6026 6125 6234 6311 6355 6476 6522 6713 7541 7560 7687 7715 7806 7827 7834 7877 7901 7911 7967	786 932 7758 1833 988 1690 1858 5117 5947 6329 696 4935 6290 6982 7001 C): 2087 6865 5 44 332 386 390 753 1097 1165 1370 1431 1951 2603 2751 2784 3282 3336 3340 3562 3566 3676 3733 3792 4270 4288 4311 4344 4554 4842 4896 4954 5047 5333 5803 5822 6516 6579 6679 7200 7457 7593 7819 7937 8096
		248 22 4021 4 5203 5 6476 6	3282
		871 2 3949 5166 6355 7877	2784
		795 1 3686 5153 6311 7834	2751 4842 8096
		385 1 3449 5127 6234 7827	2603 4554 7937
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		485 5 3372 3 4914 5 6026 6	1431 311 4 593 7
		271 317 422 454 485 3210 3221 3267 3372 4781 4827 4910 4914 5751 5790 5979 6026 7541 7560 7687 7715	1370 288 4 457 7
		117 422 3221 33 4827 43 5790 59	29 1165 270 4 200 7
		317 0 32 1 48 1 57	632 197 1 12 42 19 72
			5947 7001 53 10 3 375
		4237 0 260 3200 4770 5741 7420	858 5117 5947 290 6982 7001 865 86 390 753 10 3676 3733 379 6516 6579 667
spľ		4529 738 3188 3188 4760 7310	1858 6290 6865 386 3 3676 2 6516
tru91 mse1 ase1/asn1/vsp1 n1 p700 ATTAA		1690 5947 2969 3967 4529 823 1039 2738 4237 217 229 238 250 260 3167 3179 3188 3200 4739 4748 4760 4770 5275 5680 5699 5741 7166 7175 7310 7420 see hinli	786 932 7758 1833 988 1690 1858 5117 5947 6329 696 4935 6290 6982 7001 C): 2087 6865 5 44 332 386 390 753 1097 11 3562 3566 3676 3733 3792 427 5803 5822 6516 6579 6679 720 1876 5651 6198 7444
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			AG):
nlaiii 101 TGACCATGAT ACTGGTACTA	120	TC): ACC): C):	afili/bfri(CTTAAG): afilii(ACRYGT): agel(ACGGT): ahali/bsaHl(GRCGYQ): ahali/dral(TTTAAA): ahdi/eaml1051(GACNNN): alul(AGCT):
1 TGA ACT	>length: 8120	aatli(GACGTC): acci(GTMKAC): acli(CCGC):	/bfrI I(ACR ACCGG /bsaH I/dra eamll AGCT)
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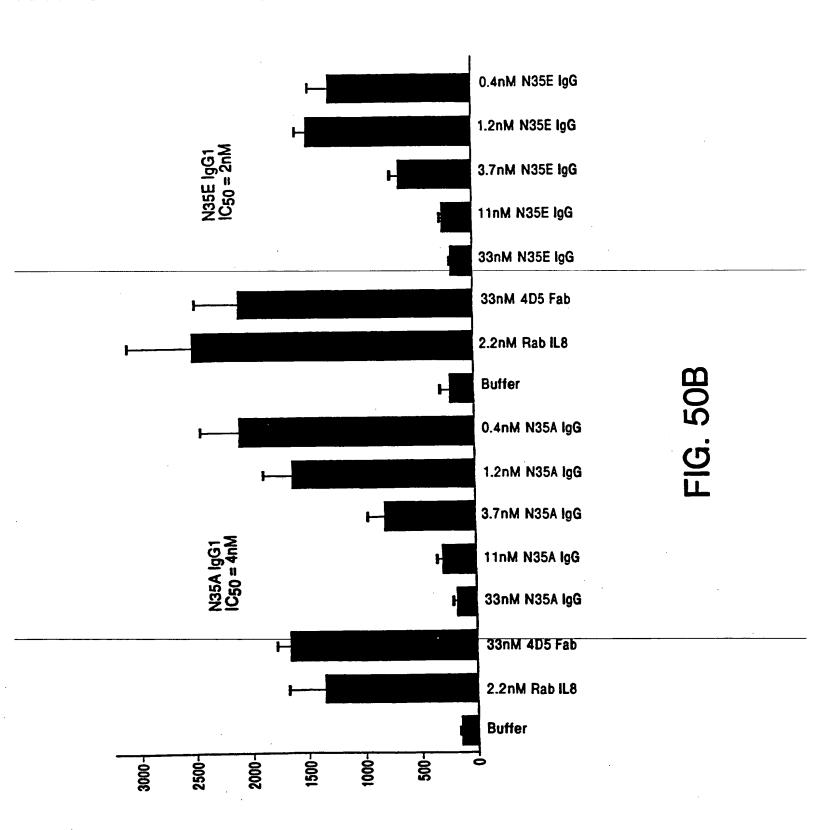




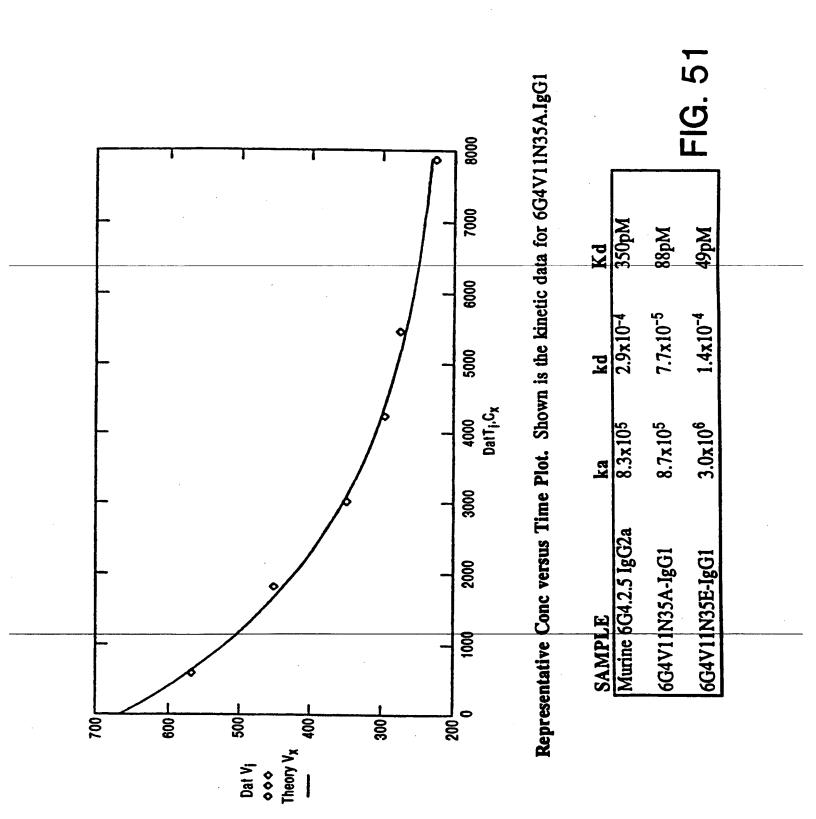


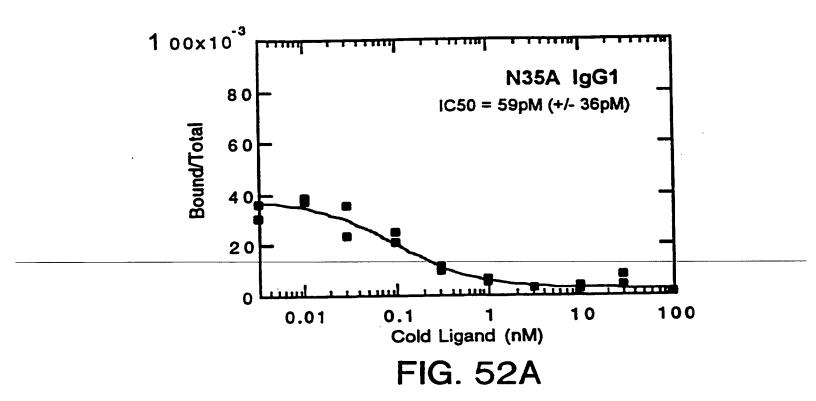


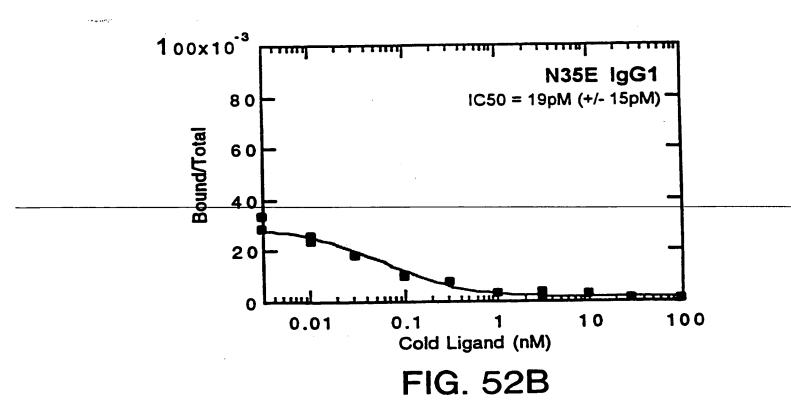
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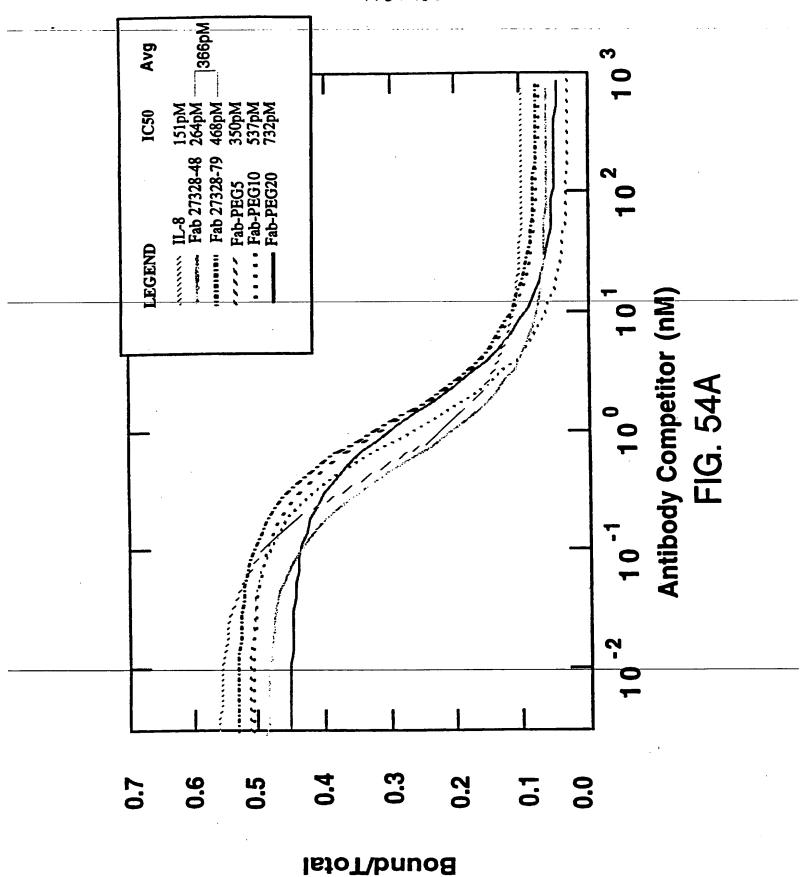


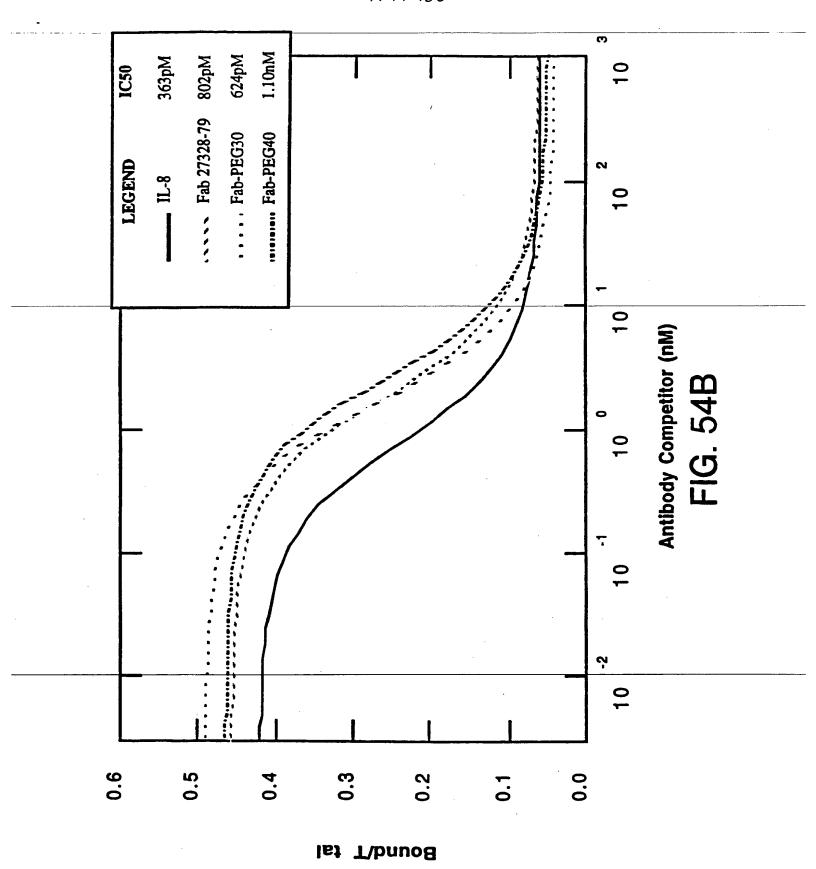


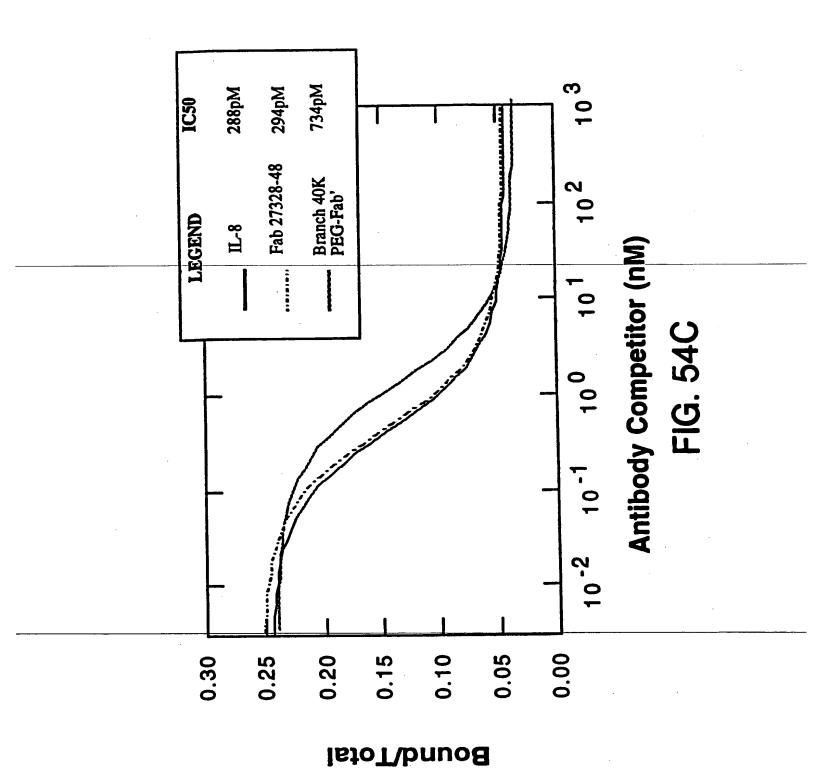
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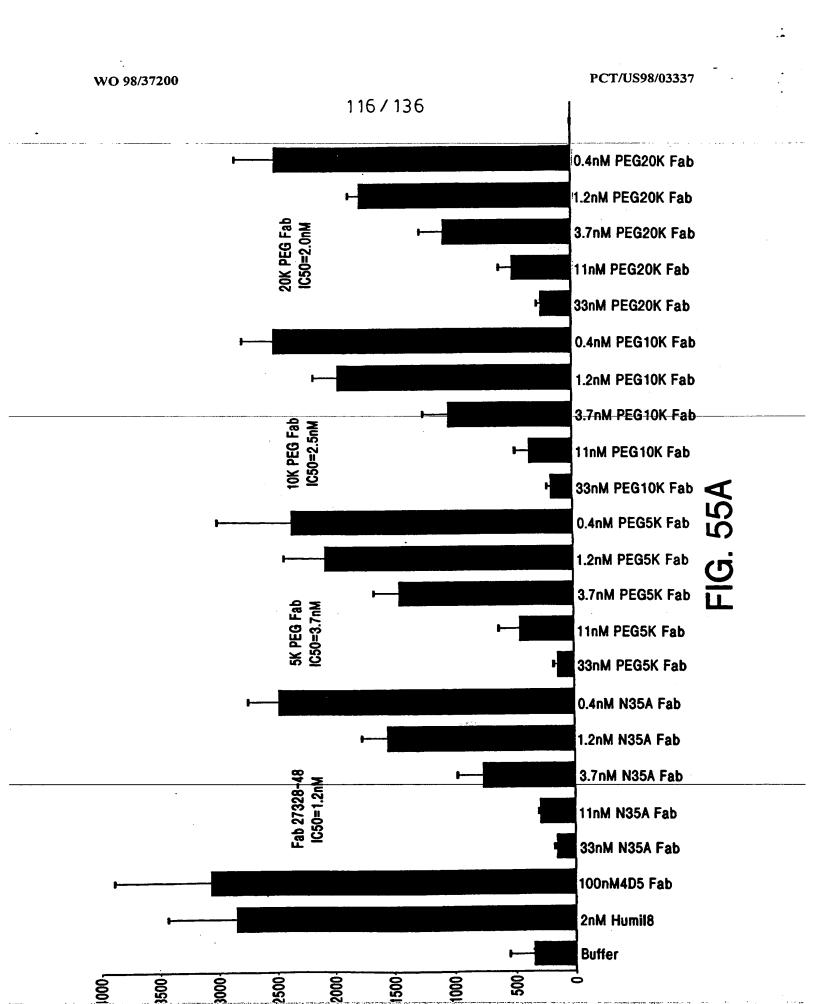
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041	AGAT		GC	AAAA	AAGA	TA	ACGZ	TG	TTG	CGC	ATC	CGAC	TC	:CA	AGTY	CGA	TCA	CGTC	AGA	
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	AAGT																			
68	F T	L	S	R	D	N	S	K	N	T	A	Y 1	Ĺ	Q	M	N	S	L	R	
1141	GCTG	AGGA	CA	CTGC	CGTC	TA	TTAC	CTG!	rgca	AG	/GGC	GAT	r An	CG	CTA	CAA	TGG	TGAC	TGG	
	CGAC'	TCCI	'GT	GACG	GCAG	TA	YTAA	SAC	ACGT	TC:	rccc	CTA	A T	\GC	GAT	GTT	ACC	ACTY	BACC	
88	A E	D	T	A	V	Y	Y	С	A	R	G_	<u>D'</u>	<u>Y</u>	R_	<u>Y</u> _	N_	<u> </u>	_D	W	
1201	TTCT	TCGA	CG	TCTG	GGGI	CA	AGG2	AAC	CCTG	GT	CAC	CGTC	r co	CTC	GGC	CTC	CAC	CAAC	GGC	
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1261	CCAT	CGG7	CT	TCCC	CCTG	GC	ACC	CTC	CTCC	AAC	GAG	CACC	T C	rgg	CCC	CAC	AGC	GCC	CCTG	
	GGTA	GCC#	<b>IGA</b>	AGGG	GGAC	CCG	TGG	<b>SAG</b>	GAGG	TT	CTC	STGG.	A GZ	ACC	CCC	GTG	TCG	CCG	<b>GAC</b>	
128	P S	v	F	P	L	A	P	S	S	K	S	T .	S	G	G	T	A	A	L	
	_																			
1321	GGCT	GCCI	rgg	TCAA	GGAC	TA	CTT	CCC	CGAA	CCC	GTY	GACG	G T	<b>GTC</b>	GTG	GAA	CTC	AGG	CGCC	
	CCGA	CGGZ	CC	AGTI	CCTC	TA	GAA	GGG	GCTT	GG	CCA	CTGC	C A	CAG	CAC	CTT	GAG	TCC	GCGG	
148	G C	L	V	K	D	Y	F	P	E	P	v	T	V	S	W	N	S	G	A	
	•																			
1381	CTGA	CCAC	CG	GCGT	GCAC	CAC	CTT	ccc	GGCT	GT	CCT	ACAG	T C	CTC	AGG	ACT	CTA	CTC	CCTC	
	GACT	GGTC	CGC	CGCA	CGTC	TG	GAA	GGG	CCGA	CA	GGA'	TGTC	A G	GAG	TCC	TGA	GAT	CAG	GGAG	
168	L T	S	G	V	Н	T	F	P	Α	V_	L	_0	S	S	G	L	Y	S	L	
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1441	AGCA	GCG1	rgg	TGAC	CGTC	SCC	CTC	CAG	CAGC	TT	GGG	CACC	C A	GAC	CTA	CAT	CTG	CAA	CGTG	
	TCGT	CGC	ACC	ACTG	GCAC	CGG	GAG	GTC	GTCG	AA	CCC	GTGG	G T	CTG	GAT	GTA	GAC	GTT	GCAC	
188	s s	V	V	T	v	P	S	s	S	L	G	T	0	T	Y	I	С	N	V	
200		•	•		-	-	-	_	_	_		_	-							
1501	AATC	ACAI	AGC	CCAG	CAAC	CAC	CAA	GGT	CGAC	AA	GAA	AGTT	G A	GCC	CAA	ATC	TTC	TGA	CAAA	
1301	TALF	העיטעו	CCC	GGTC	GTT	STC	GTT	CCA	GCTG	TT	CTT	TCAA	CT	CGG	GTI	TAG	AAC	CACT	GTTT	
208	N H	K	P	S	N	T	ĸ	v	D	ĸ	K	V	E	P	K	S	С	D	K	
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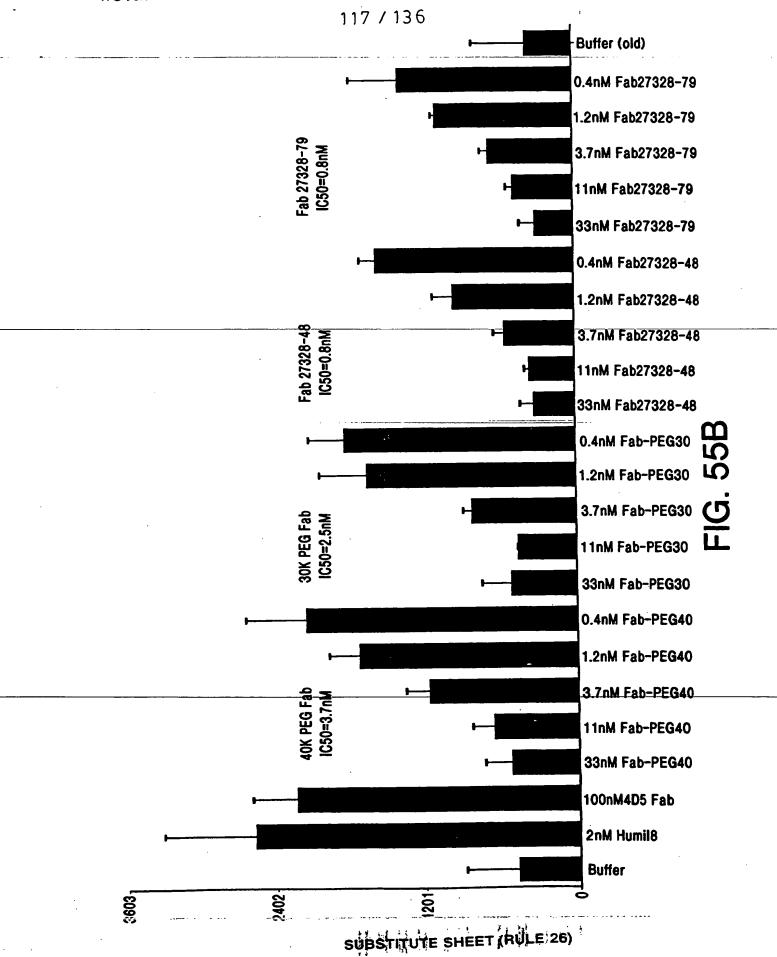


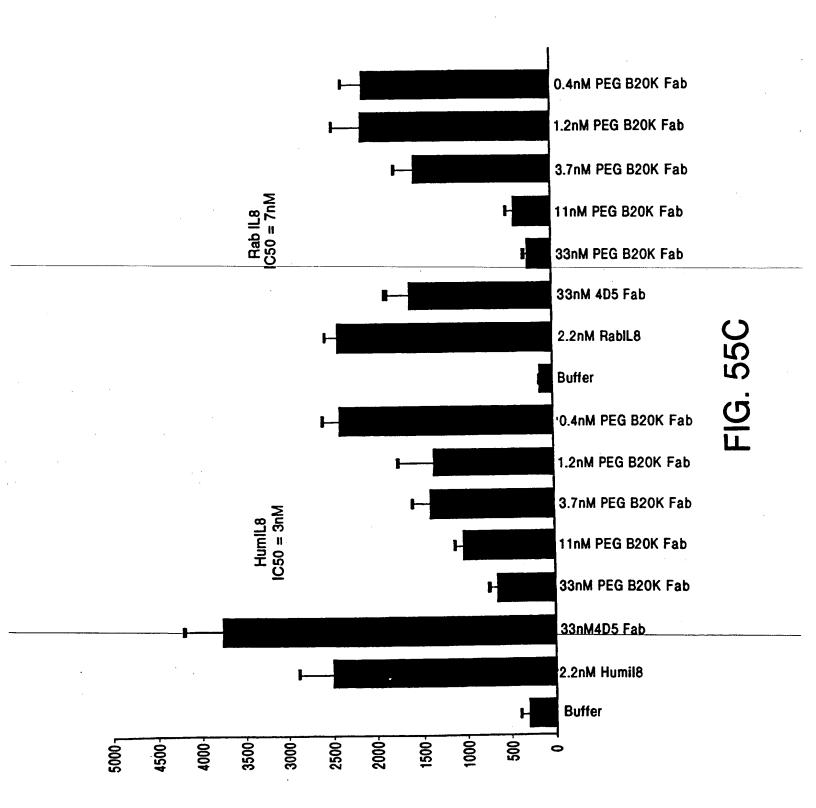


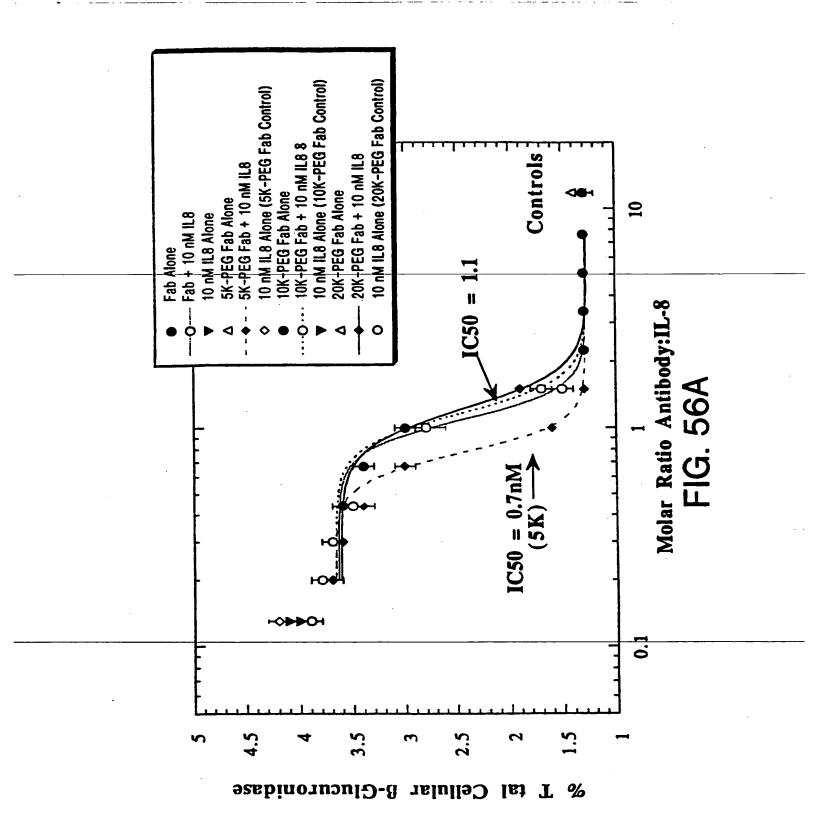


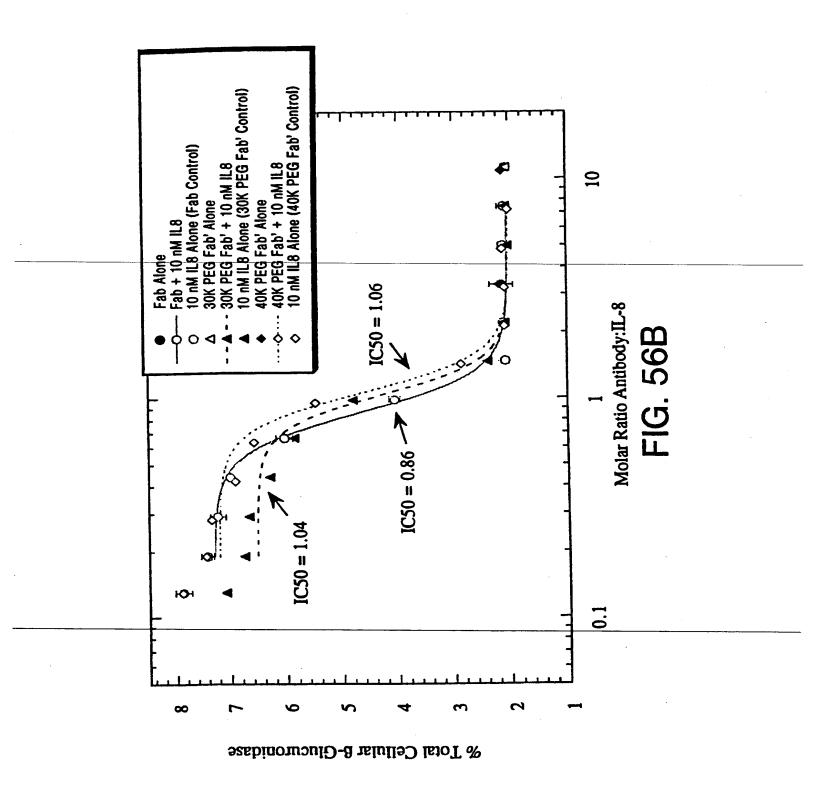


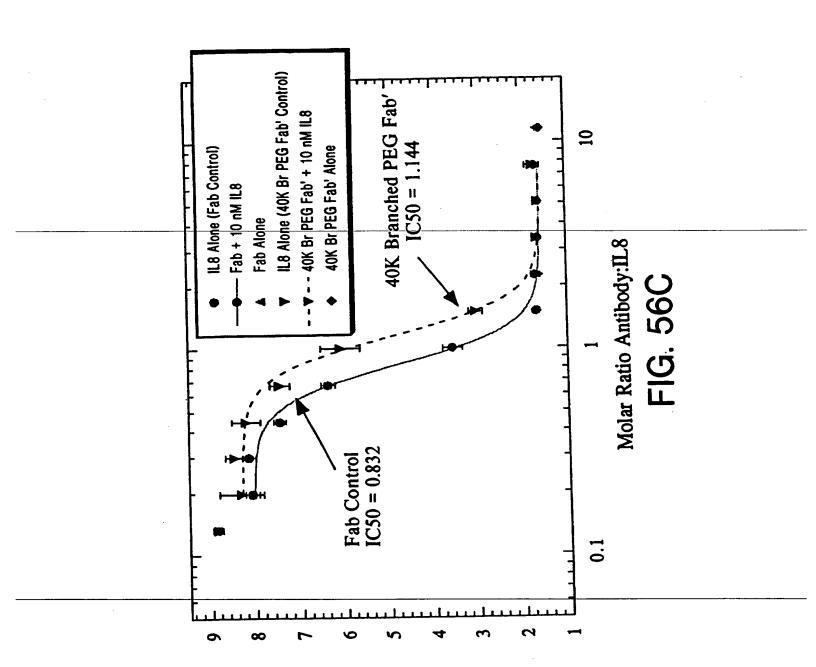
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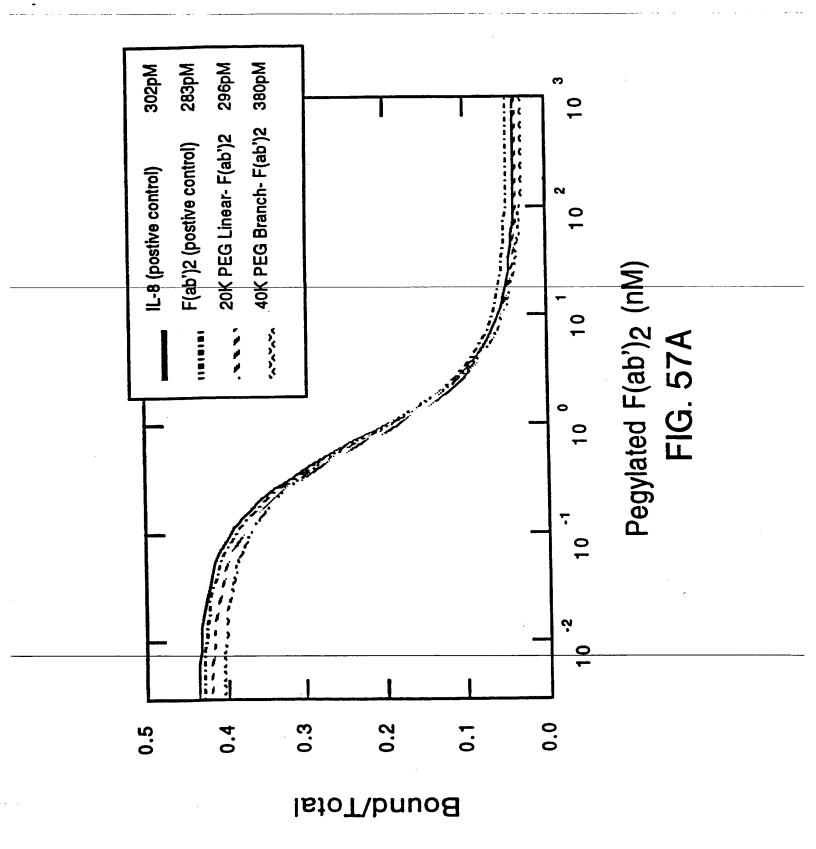


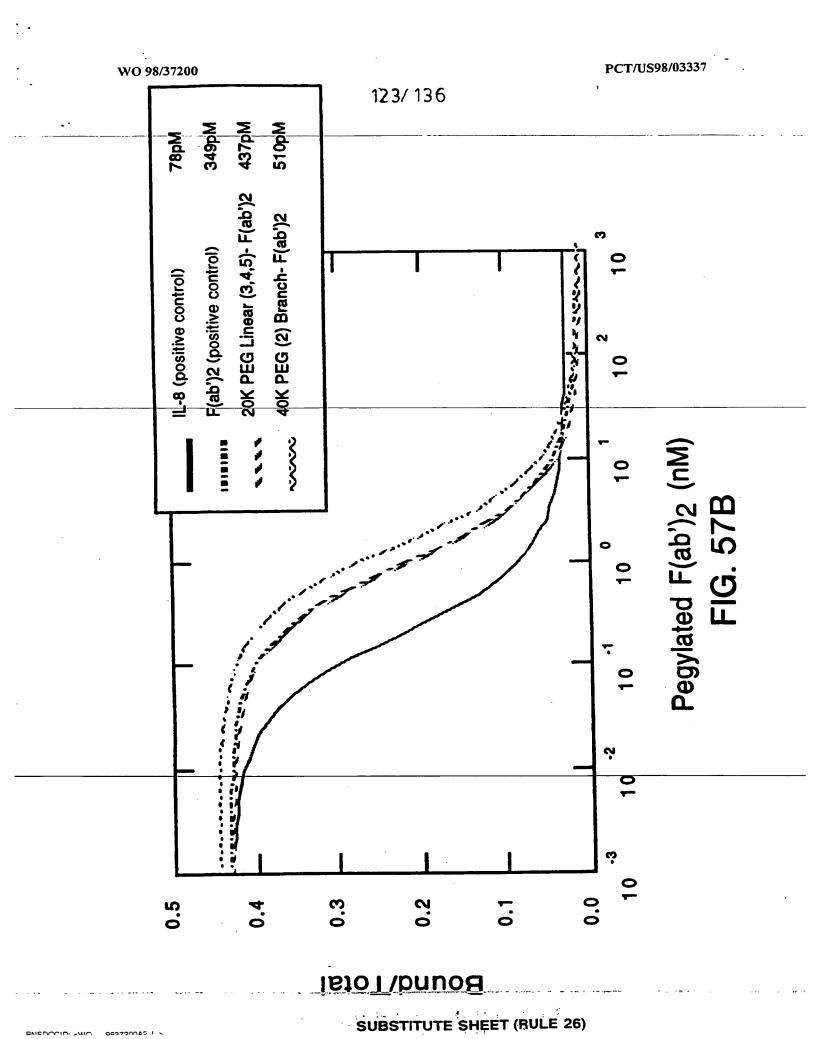




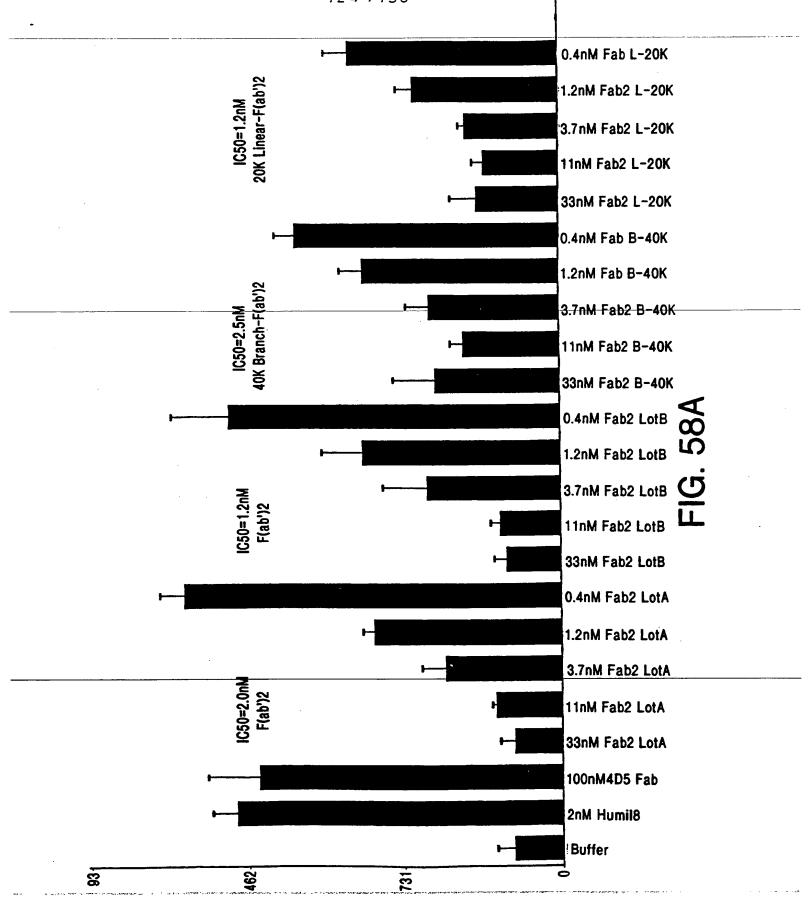


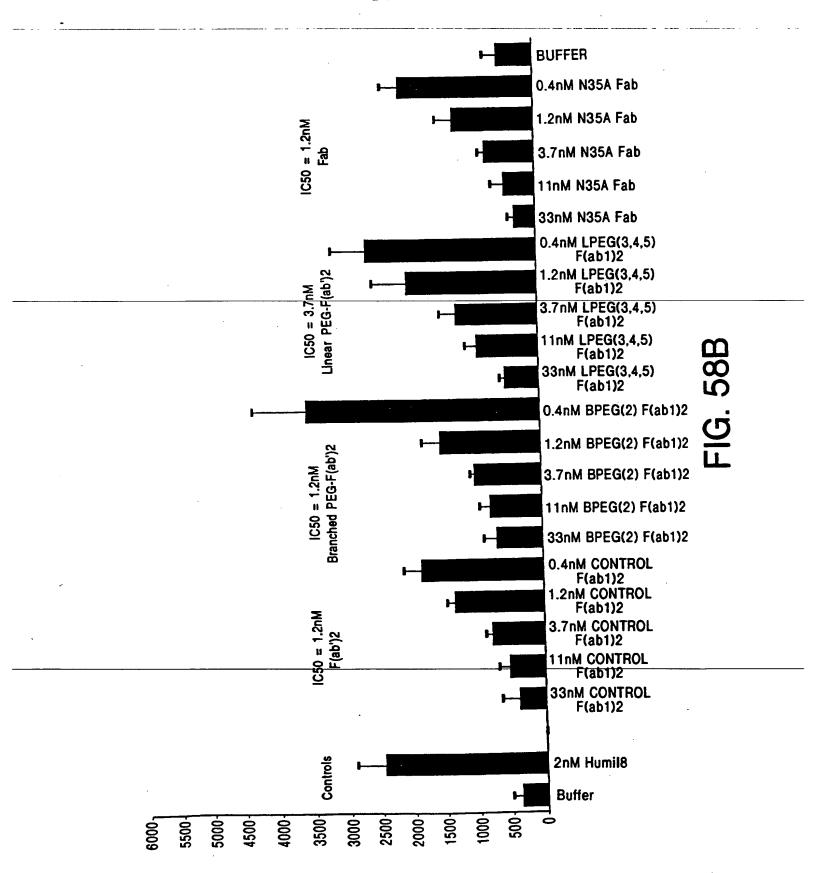
% Total Cellular B-Glucuronidase Activity



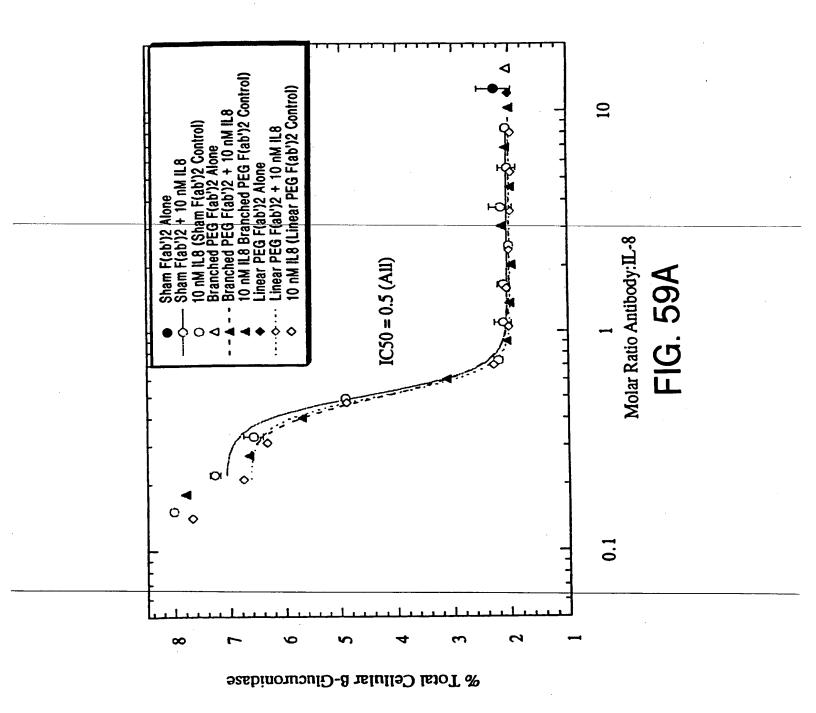


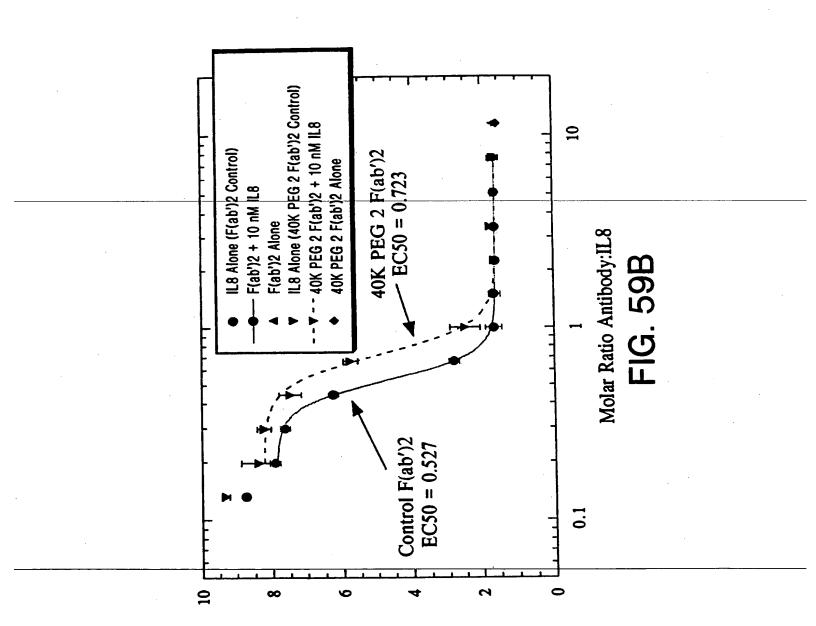
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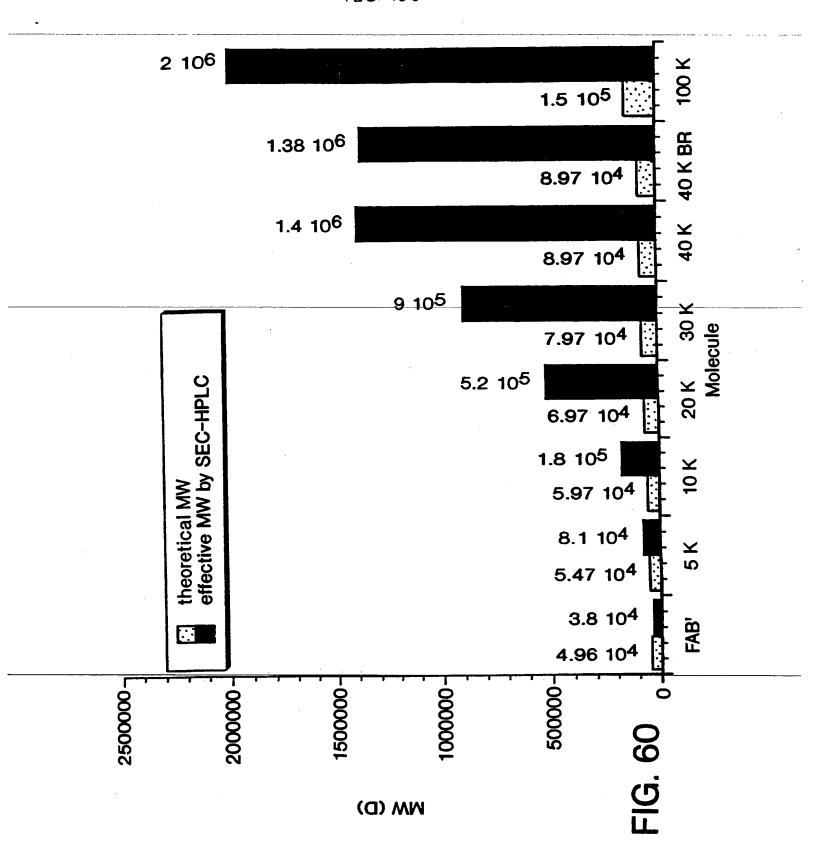
SUBSTITUTE SHEET (RULE 26)





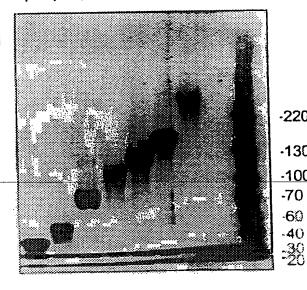
% Total Cellular B-Glucuronidase Activity

128/136



-5K -10K -20K -30K -40K -40K branch -100K -Fab

Reduced

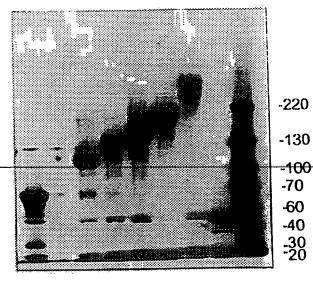


-220 -130 -100 -70 -60

FIG. 61A

-20K -30K -40K -40K pranch

Non-Reduced



<del>-100</del> -70

-60 -40

FIG. 61B

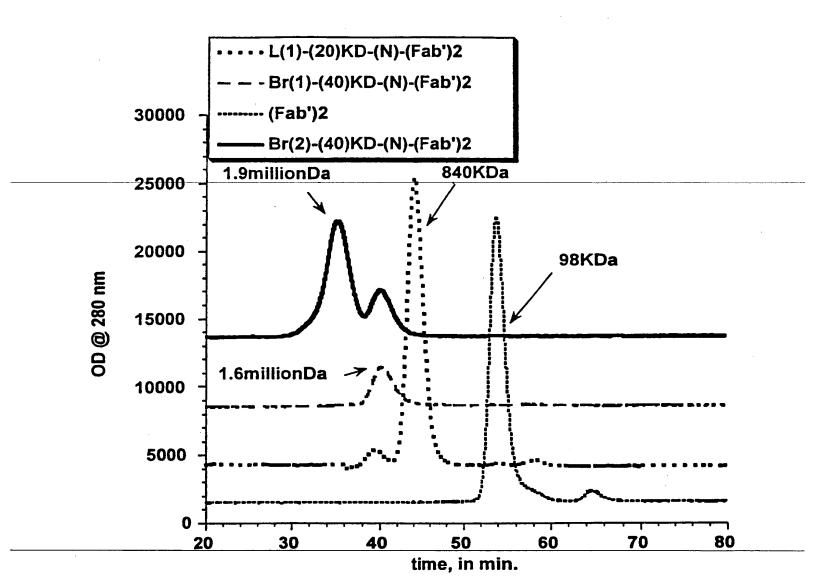


FIG. 62

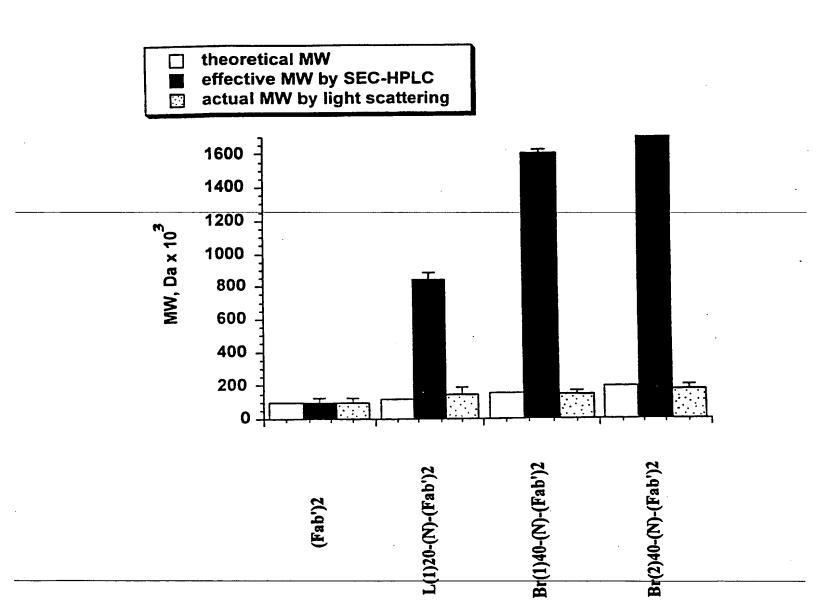


FIG. 63

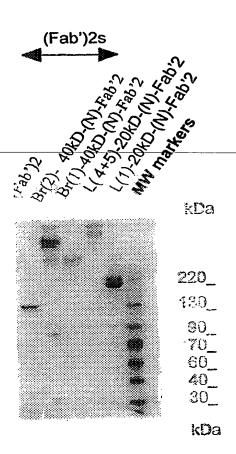
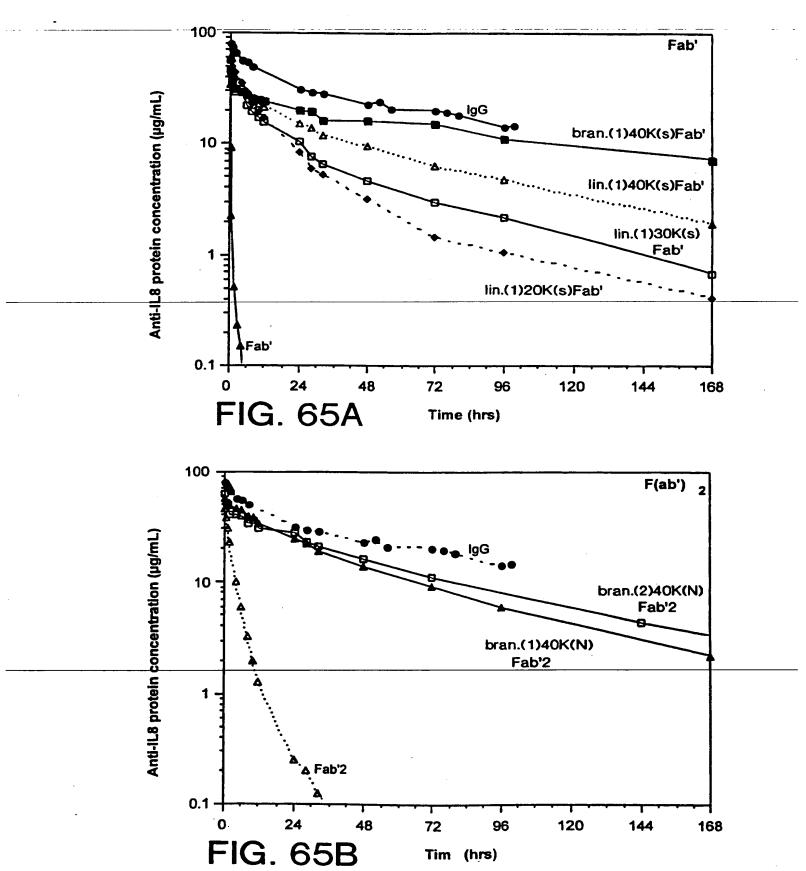


FIG. 64



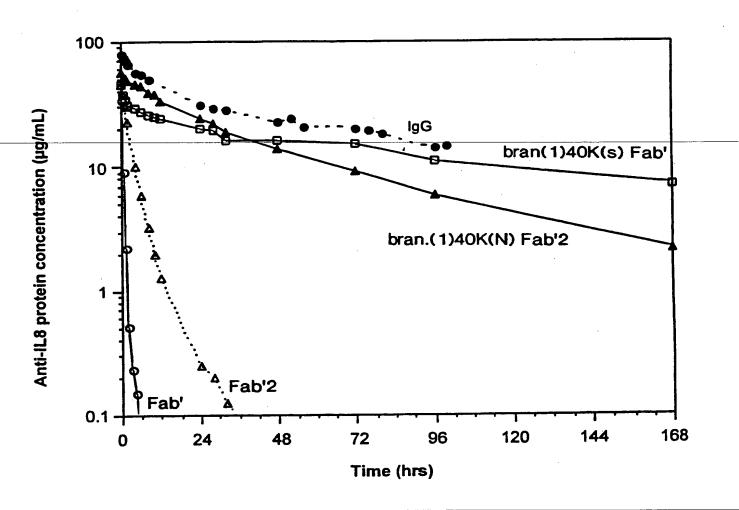
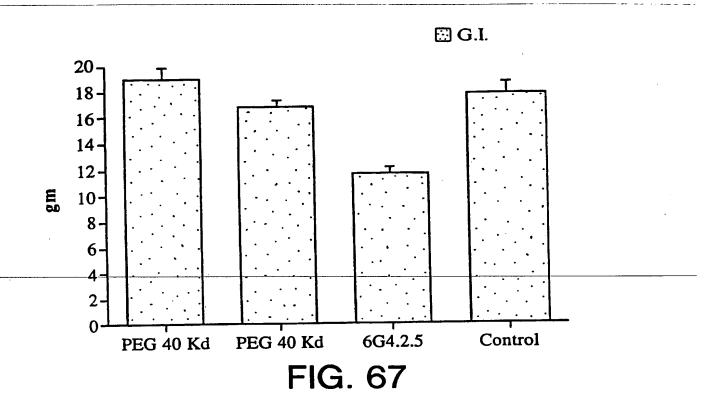
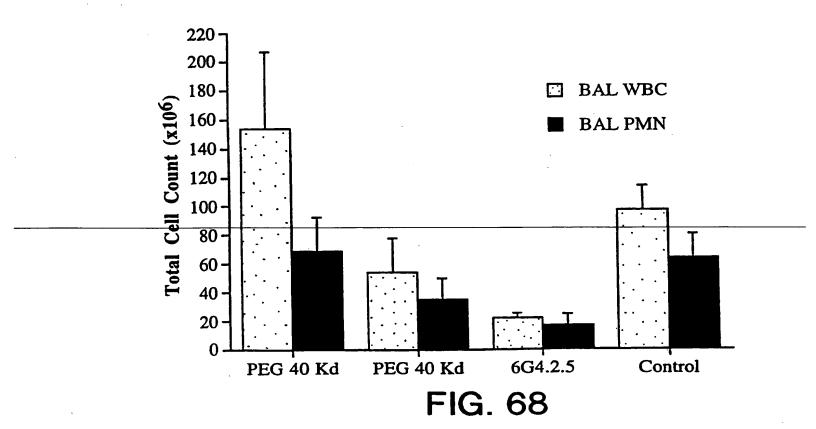


FIG. 66





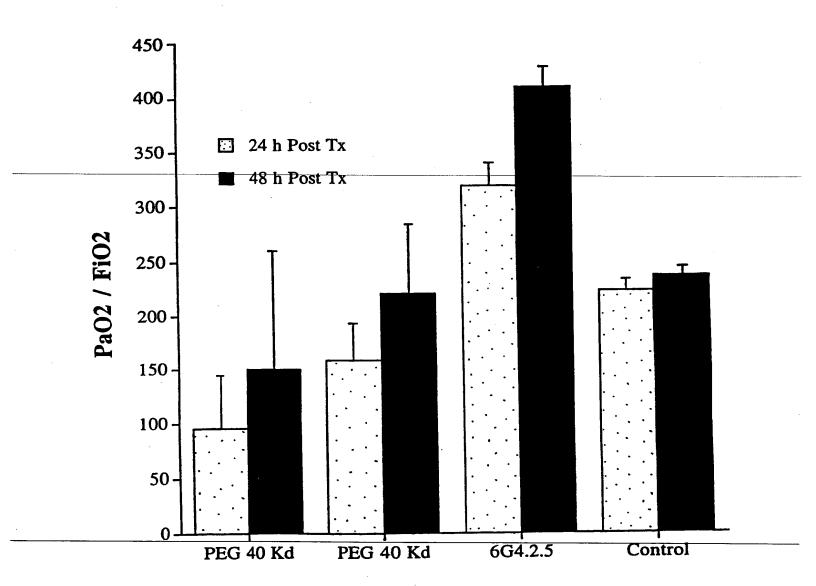
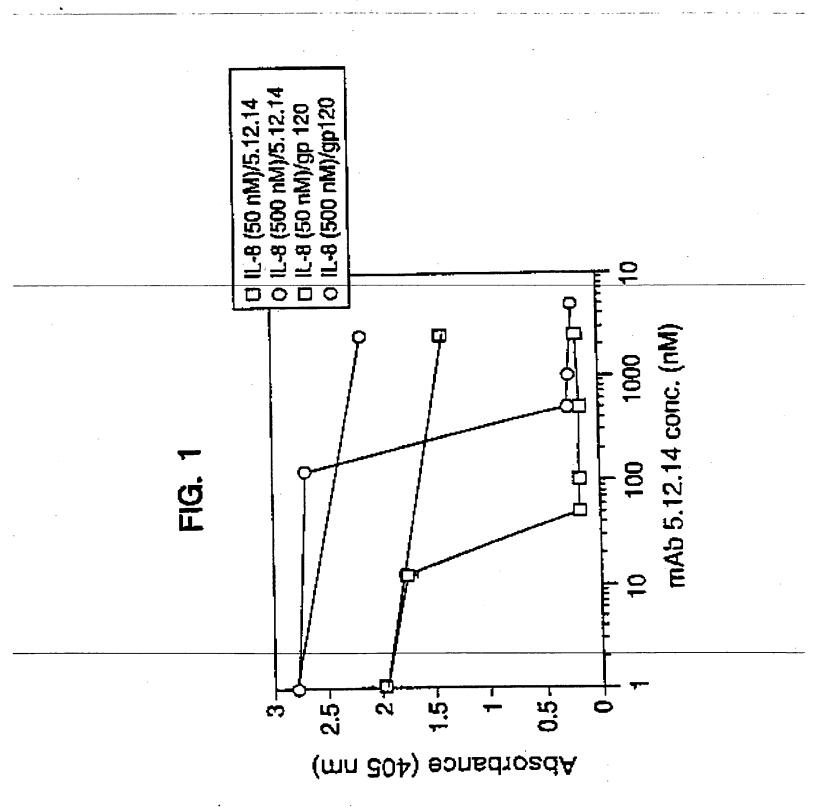
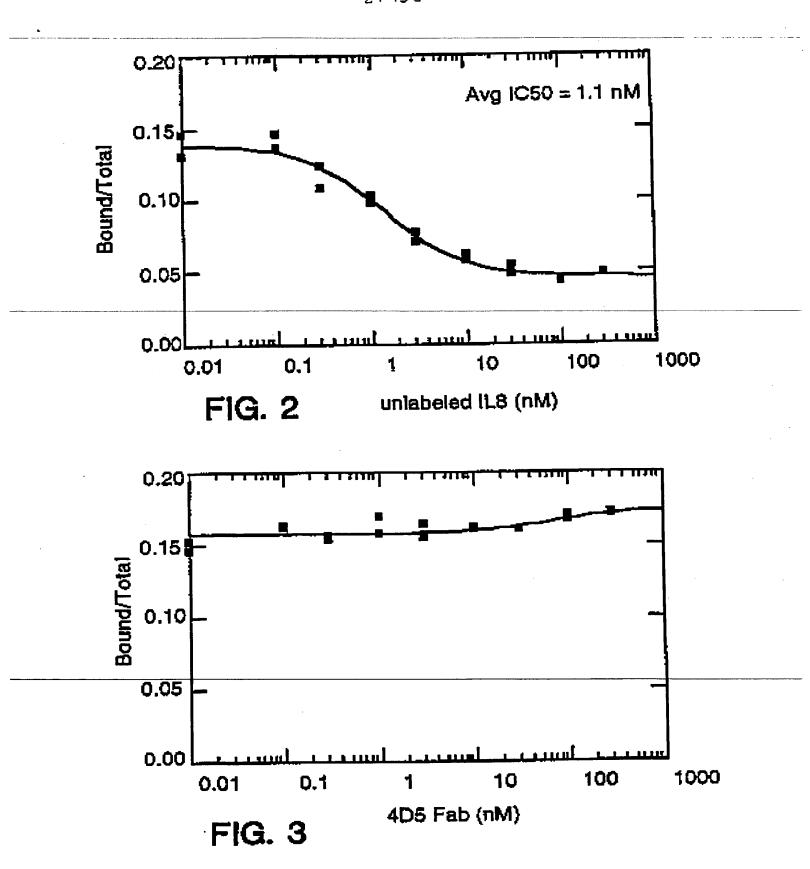
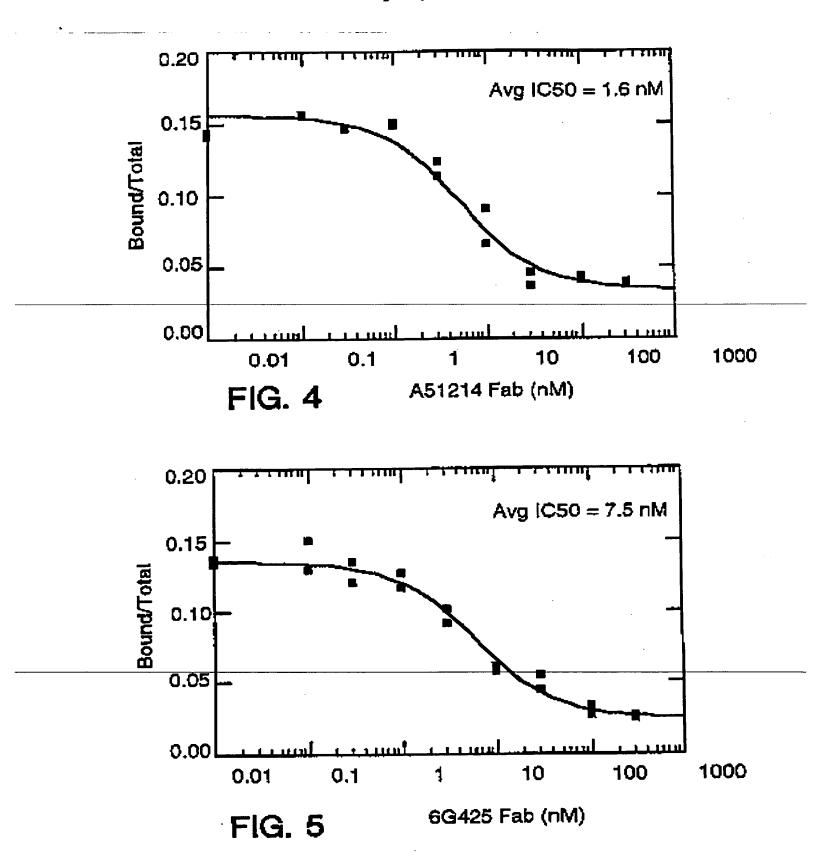


FIG. 69







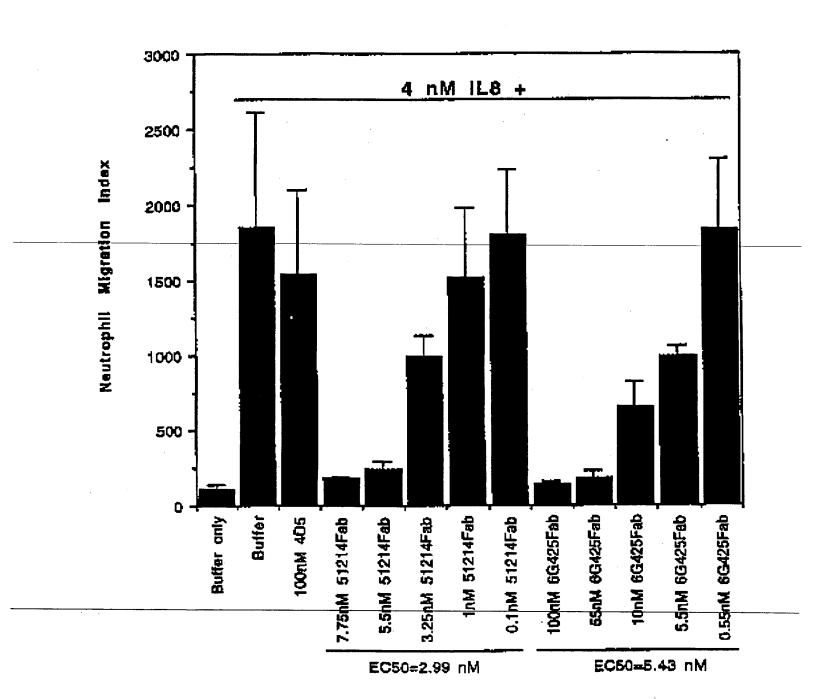


FIG. 6

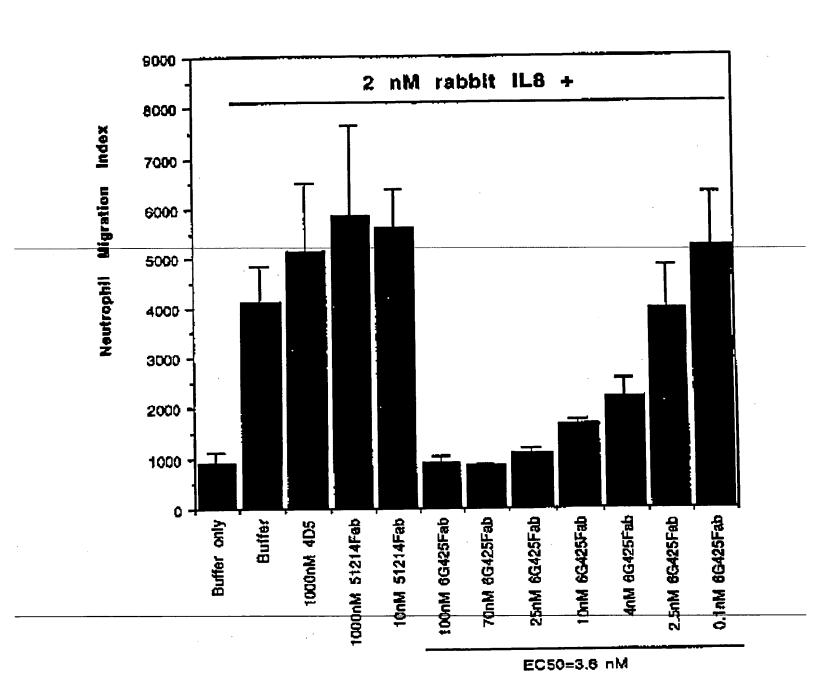
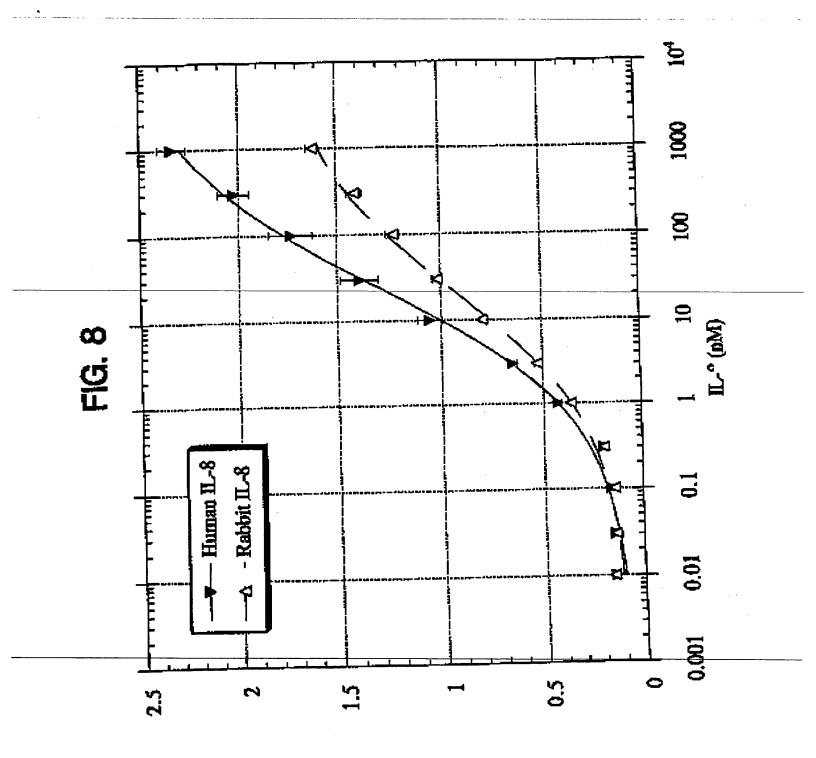
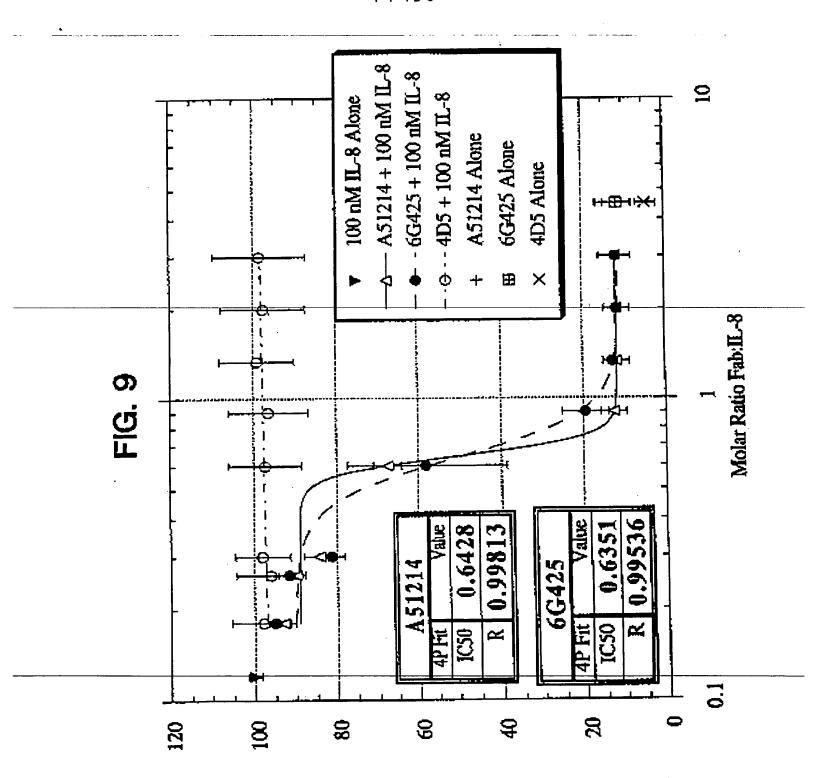


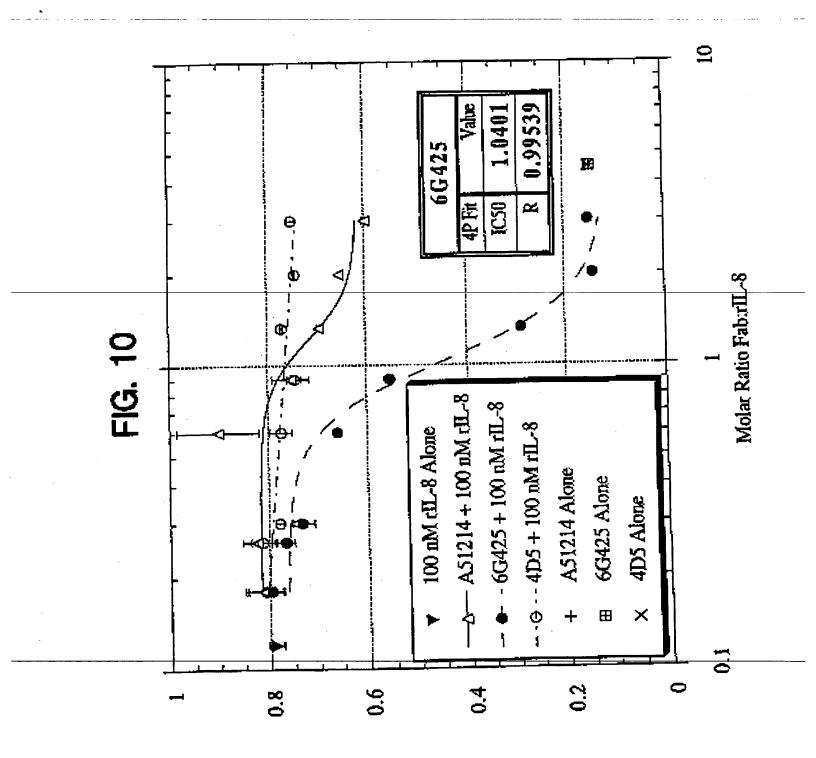
FIG. 7



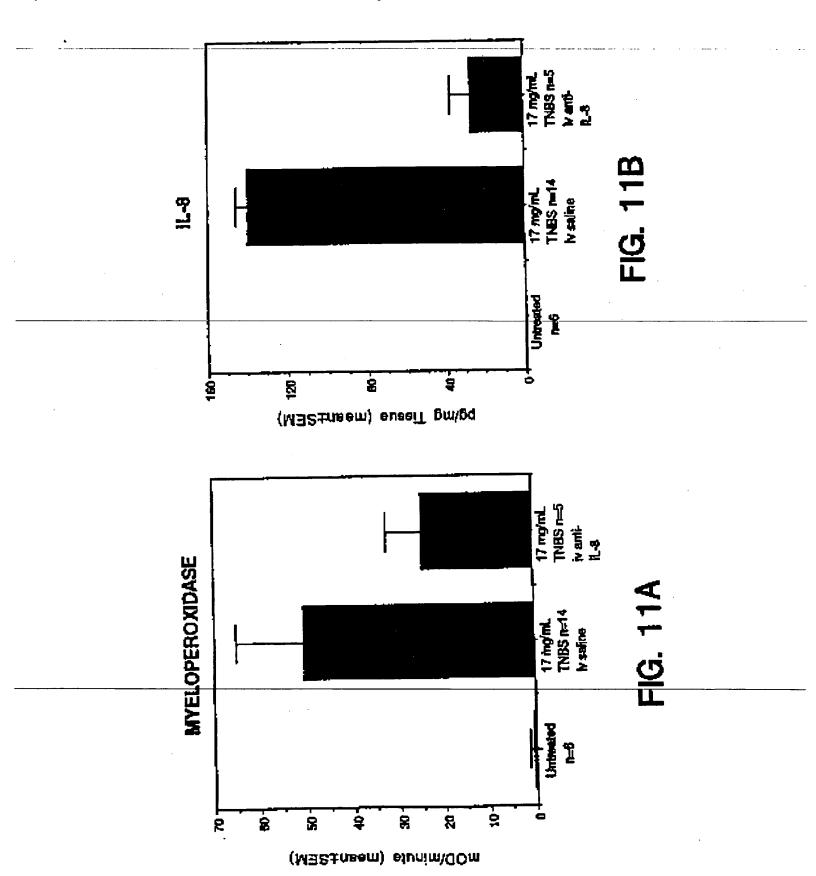
Absorbance (405 nm)

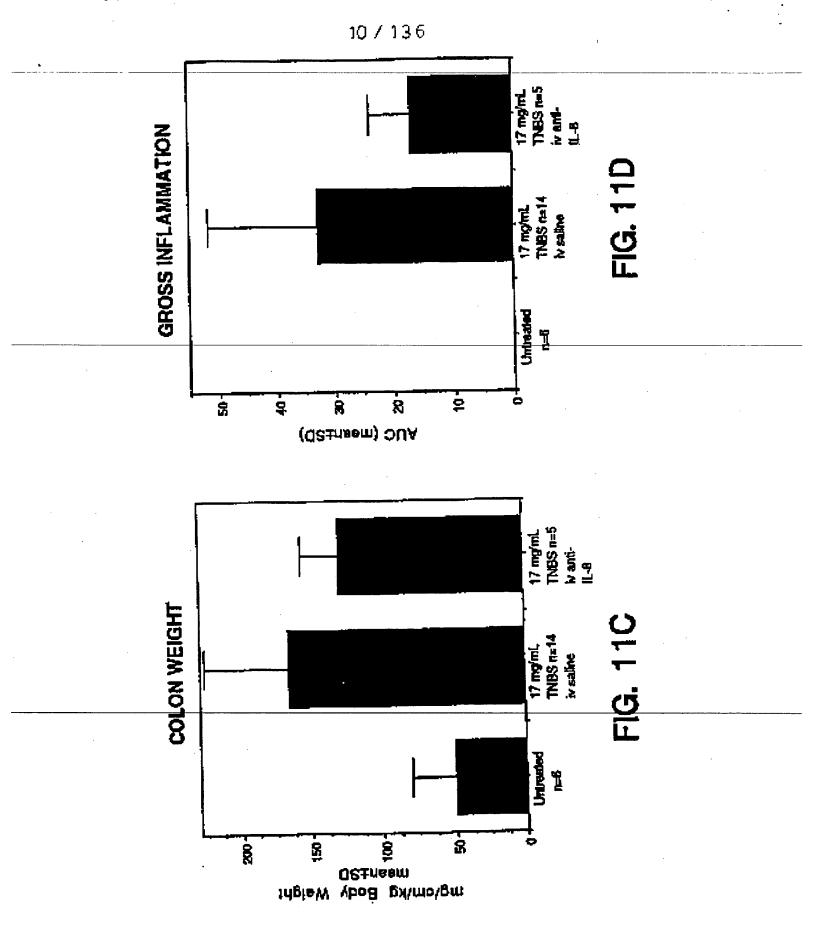


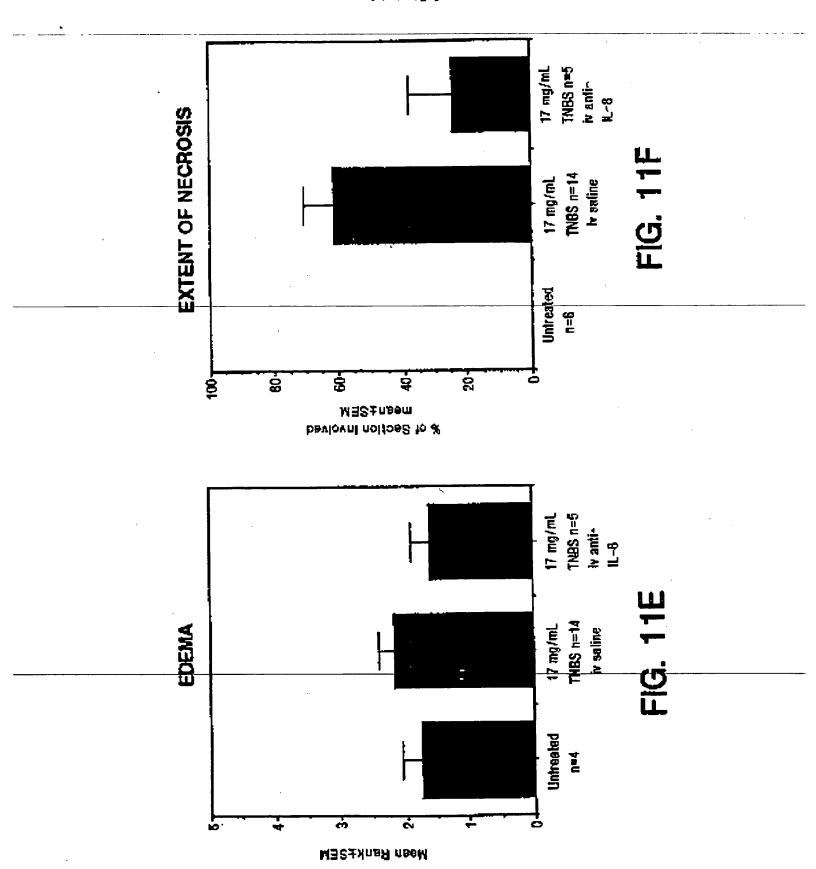
% IL-8-Stimulated Hastase Release



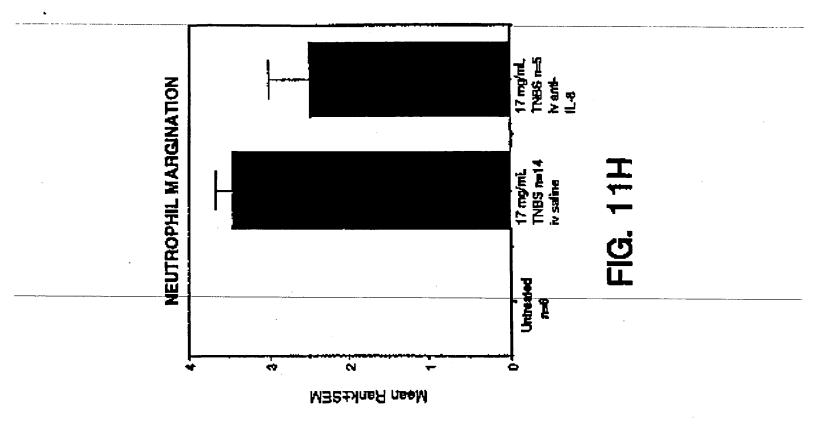
Absorbance (405 nm)

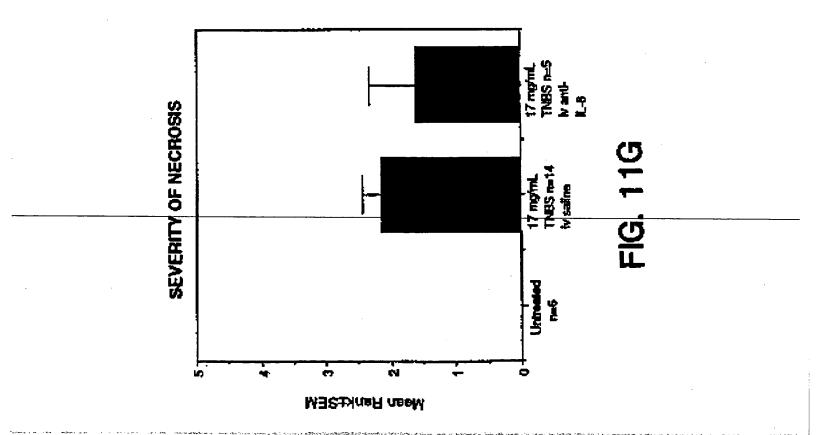


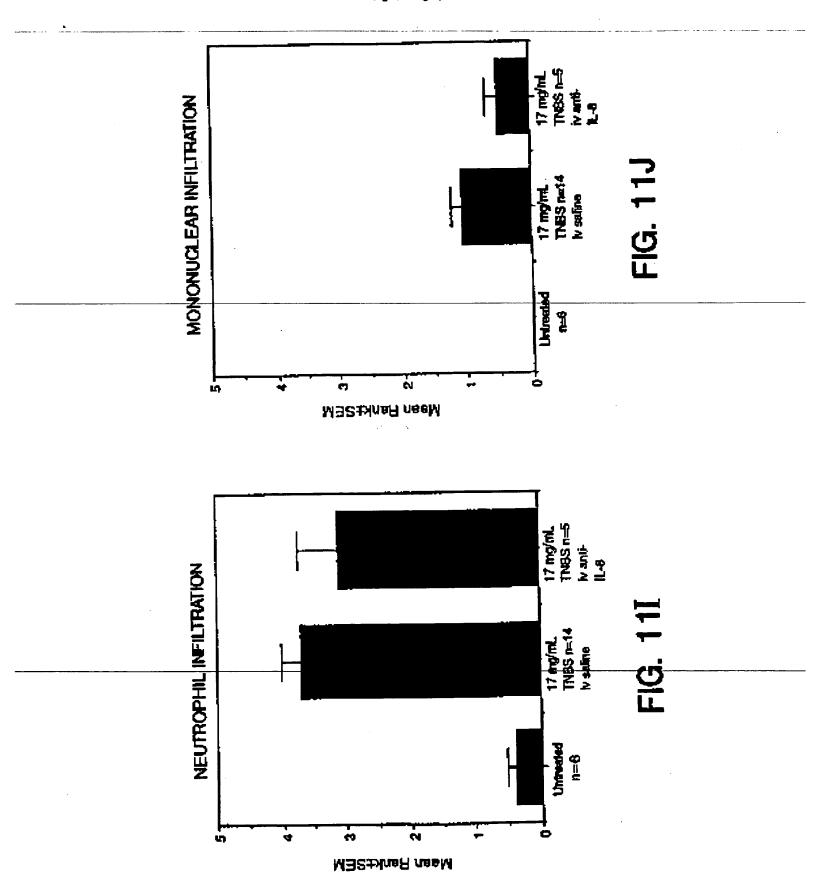




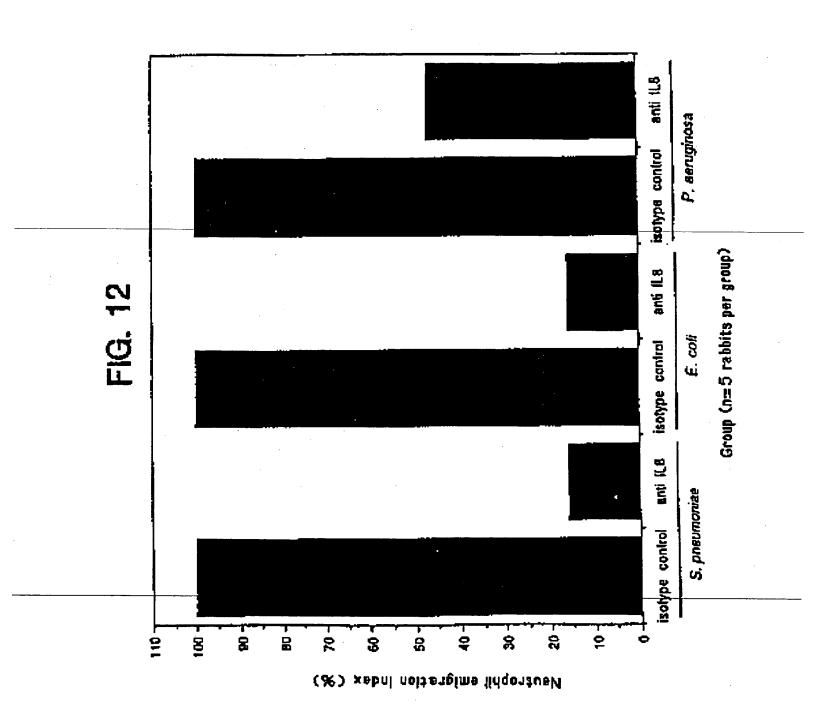
SUBSTITUTE SHEET (RULE 26)







SUBSTITUTE SHEET (RULE 26)



Light Ch	ain Primers:		40	
MKLC-1,	22mer	FIG.	13	
51	CAGTCCAACTGTTC	AGGACGC	2C 3'	
MKLC-2,	22mer			
5'	GTGCTGCTCATGCT	GTAGGT	3'	
MKLC-3,	23mer			
5 '	GAAGTTGATGTCTT	GTGAGT	G <b>C</b>	3 '
Heavy C	hain Primers:			
IGG2AC-	1, 24mer			
, <b>5</b> '	GCATCCTAGAGTC	CCGAGG	AGCC	3 '
IGG2AC-	2, 22mer			
5 '	CACTGGCTCAGGG	AAATAAC	CC 3'	
IGG2AC-	3, 22mer			
E 1	▗ ▗▗▄᠉Ċ᠉ĠĊͲĠĠĠĨŶŶ	GGTGTGC	CAC 3'	

SL001B 37 mer

### FIG. 14

Light chain forward primer

SL001A-2 35 mer

5' ACAAACGCGTACGCT GACATCGTCATGACCCAGTC 3'

T T T

A

Light chain reverse primer

5' GCTCTTCGAATG GTGGGAAGATGGATACAGTTGGTGC 3'

SL002B 39 mer

5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC 3'

T C
G
A

Heavy chain reverse primer

SL002B 39-MER

5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC 3'

T A

Heavy chain forward primer

AGGAGA CAGGGTCAGC ICCTCT GTCCCAGTCG G D R V S	GTATCA ACAGAAACCA CATAGT TGTCTTTGGT Y Q Q K P	GGTACAGYGG AGYCCCYGAT CCATGTCACC TCAGGGACTA Y S G V P D * *	CCATCAGCCA TGTGCAGTCT GGTAGTCGGT ACACGTCAGA I S H V Q S	Arcticaca Gricgerch Taggagaga Caagccagga P L T F G P	INGTATC CANCITCCCA	9
MC ATGTCCACAT CAGTAGGAGA	GICACCIGCA AGGCCAGTCA GAATGTGGGT ACTAATGTAG CCTGGTATCA ACAGAAACCA CAGTGGACGT TCCGGTCAGT CTTACACCCA TGATTACATC GGACCATAGT TGTCTTTGGT  V T C K A S O N V G T N V A N Y Q Q K P  CDR #1	GATITACTCG TCATCCTACC GGTACAGTGG CTAAATGAGC AGTAGGATGG CCATGTCACC I Y S S Y R Y S G	AT TICACTUTCA FA AAGIGAGAGT FT LT	TATAACATCT ATATTGTAGA Y N I Y  * * * * *	ACGGCTGAT GCTGCACCAC CAACTGTATC TGCCCGACTA CGACGTGGTG CTTGACATAG R A D A A P P T V S	FIG. 16
IGACACAGTC TCAAAAATTC ACTGTGTCAG AGTTTTTAAG T Q S Q X F	AGGCCAGTCA GAATGTGGGT TCCGGTCAGT CTTACACCCA A S O N V G A * * * * * * * * * * * * * * * * * * *		GCAGTGGATC TGGGACAG CGTCACCTAG ACCCTGTC' S G S G T D	CIGICAG GACAGIC C Q	TGGAGTTGAA ACGGGCTGAT ACCTCAACTT TGCCCGACTA E L K R A D	
1 GACATTGTCA TGACACAGTC CTGTAACAGT ACTGTGTCAG 1 D I V M T Q S	61 GTCACCTGCA 2 CAGTGGACGT 2 21 V T C K	121 GGGCAATCTC CTAAAGCACT CCCGTTAGAG GATTTCGTGA 41 G Q S P K A L	181 CGCTTCACAG GCGAAGTGTC ( 61 R F T G	241 GAAGACTIGG CAGACTATTT CTICTGAACC GICTGAIAAA 81 B D L A D Y F	301 GGGACCAAGC CCCTGGTTCG	BSEBI 361 CCATTCGAA GGTAAGCTT 121 P F E

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		TOTTTGCGCA TG													
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				CDR #1											
121		TGGGTTCGCC AG													
	ACCOTACAGA	ACCCAAGCGG TC	TGAGGTCC	GTTCTCGGAC	CTCAACCAGC										
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		CDR #2													
		<b>42</b> - 1 · 1 · 1													
241	AGACAATGCC	AAGAACACCC TG													
	TCTGTTACGG	TTCTTGTGGG AC	TODAEOTA	TTACTCGTCA											
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301	CATGTTTTAC	TGTGCAAGAG CCGACACGTTCTC GGG	CTCATTAG CACTA AUC	TTCGGCTACT	TOGTTIGGTT	TGACCCCGGT									
			GAGIAAIC L I S	AAGUCGAIGA S A T	W F G Y	W G O									
93	M F Y	CARA,	<del></del>	* * *	<del>1 + + +</del> +	. " • •									
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501		CAGTGACAGA GA													
113	g T L	V T V S	AAK	A T T	PSVY										

VL.front	31-MER		
5' ACAA <u>ACGCGT</u> VL.rear 31-MI	qacgot <u>gatatc</u> gtcatgacag er	3 '	
5' GCAGCATCAC	CTC <u>TTCGAA</u> GCTCCAGCTTGG	3'	
VH.front.SPE	21-MER		
5' CCACTAGTAC	CGCAAGTTCACG	3'	
VH.rear 33-M	ER		
5 GATGGGCCC	TTGGTGGAGGCTGCAGAGACAG	TG	3

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	GI	ÇTT	TGG	TC	CCGT	TAG	igg.	ATT	TCG:	TGAC	TAJ	Yra <i>f</i>	RGC	A	GTAG	Gan	3GC	CAT	GTC	ACCT	
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SUBSTITUTE SHEET (RULE 26)

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- 661 ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGGA ACACCAAGGT GGACAAGAAA TGGGTCTGGA TGTAGACGTT GCACTTAGTG TTCGGGTCGT TGTGGTTCCA CCTGTTCTTT 198 T Q T Y I C N V N H K P S N T K V D K K
- 721 GTTGAGCCCA AATCTTGTGA CAAAACTCAC ACATGA CAACTCGGGT TTAGAACACT GTTTTGAGTG TGTACT 218 V B P K B C D K T H T O

FIG. 20B

Light Ch	ain Primers:	
MKLC-1,	22mer	
5 '	CAGTCCAACTGTTCAGGACGCC 3'	
MKLC-2,	22mer	
5'	GTGCTGCTCATGCTGTAGGTGC 3'	
MKLC-3,	23mer	
5 t	GAAGTTGATGTCTTGTGAGTGGC	3 •
Heavy Ch	nain Primers;	
IGGZAC-1	., 24mer	
5 1	GCATCCTAGAGTCACCGAGGAGCC	3 '
IGG2AC-2	2, 22mer	
5 '	CACTGGCTCAGGGAAATAACCC 3 '	

GGAGAGCTGGGAAGGTGTGCAC 3'

IGG2AC-3, 22mer

Light chain forward primer

6G4.light.Nsi 36-MER

5' CCAATGCATACGCT GAC ATC GTG ATG ACC CAG ACC CC 3'
T T T T
A A

Light chain reverse primer

6G4.light.Mun 35-MER

5' AGA TGT CAA TTG CTC ACT GGA TGG TGG GAA GAT GG 3'

#### Heavy chain forward primer

6G4.heavy.Mlu 32-MER

5' CAAACGCGTACGCT GAG ATC CAG CTG CAG CAG 3'
T C

Heavy chain reverse primer

SL002B 39-MER

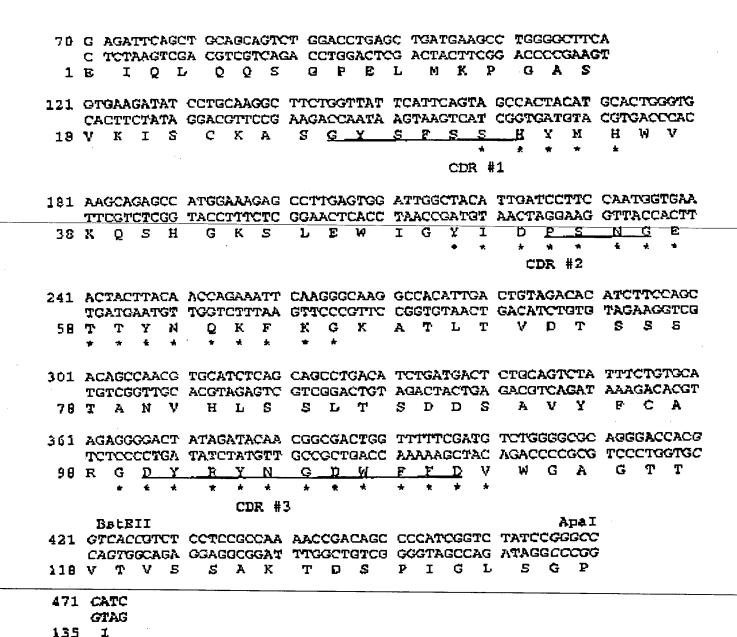
5 CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTTTGGC 3

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70 G ATATOGTGAT GACACAGACA CCACTCTCCC TGCCTGTCAG TCTTCGAGAT C TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA 1 D I V M T Q T P L S L P V S L G D 121 CAGGCCTCCA TCTCTTGCAG ATCTAGTCAG AGCCTTGTAC ACGGTATTGG AAACACCTAT GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGGAACATG TGCCATAACC TTTGTGGATA 18 Q A S I S C R S <u>S O S L V H G I G N T Y</u> CDR #1 181 TTACATYGGT ACCYGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATGTA CAAAGTTTCC AATGTAACCA TGGACGTCTT CGGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTTCAAAGG 3B L H W Y L Q K P G Q S P K L L I Y K V S * * ♥. CDR #2 241 AACCGATTIT CIGGGGTCCC AGACABGTTC AGTGGCAGIB GATCAGGGAC AGATTICACA TTGGCTAAAA GACCCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT 58 N R P S G V P D R F S G S G T D F T 301 CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAGTACA GAGTCCTAGT CGTCTCACCT CCGACTCCTA GACCCTGAAA TAAAGACGAG AGTTTCATGT 78 L R I S R V E A E D L G L Y F C S Q S T CDR #3 361 CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC TGAAACGGGC TGATGCTGCA GTACAAGGCG AGTGCAAGCC ACGACCCTGG TTCGACCTCG ACTTTGCCCG ACTACGACGT 98 H V P L T F G A G T K L E L K R A D A A 421 CCARCTGTAT CCATCTTCCC ACCATCCAGT GAGCAATTGA GGTTGACATA GGTAGAAGGG TGGTAGGTCA CTCGTTAACT 118 PTVS I PPPSSEQLK



5' CTTGGTGGAGGCGGAGGAGACG 3'

Mutagenesis Primer for 6G425VL

DS/VF 38MER

5' GAAACGGGCTGTTGCTGCACCAACTGTATTCATCTTCC 3'

SYN.BstEII 31 MER

5' GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 3'

SYN.Apa 22 MER

5' CTTGGTGGAGGCGGAGAGACG 3'

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661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGAA CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCT		
CAG		
661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGG CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTCTCGAA GTTGTCCCC 198 A C E V T H Q G L S S P V T K S F N R G		
GPA PA		
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661 198	72: 21	

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367					TATC																
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421	GT	CAC	CGT	CT.	CCTC	CGC	CTC	CAC	CAA	.GGGC	CC	ATC	GGT	T	TCCC	CCT	GGC	ACC	CTC	CTCC	•
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FIG 28A SUBSTITUTE SHEET (RULE 26)

CAAGGTGGAC

GTICCACCTG la; TICGGCACC AGACCIACAT CIGCAACGIG AATCACAAGC CCAGCAACAC GGTCGTTGTG 2 ৸ 721 AAGAAAGITG AGCCCAAATC TTGTGACAAA ACTCACACAT GA TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CT TTAGTGTTCG Н Щ GACGITGCAC × z U Ω U TTCTTTCAAC TCGGGTTTAG AACCCGTGGG TCTGGATGTA Ø U 218 **661** 198

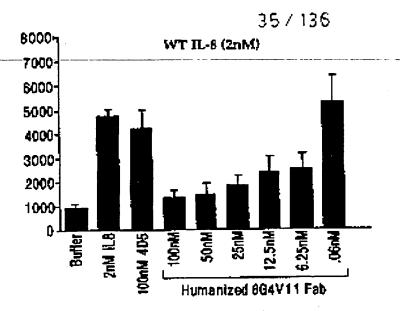
28B

#### Variable Light Chain Domain

		10	20	abode 30	40	
6G425	DIVNYOT # #	Plalpvsla # ## #	edoabiber!	egslykgig <b>nt</b>	ylhnyldkræð: #       #	
F(ab)-1			DRVTITCR	BOSTAHBH###	KTHMAÖÖKEGK:	PRELIY
humĸI	DIOMOS	Peeleasvo	DR <b>VT I</b> TCRI	es <b>x</b> ti5X	adancolmuta Adamona	APKLLTY
				398F4C::::::::	=	
			•	6e++++++	+++	
				L1		
	50	60	70	BD	90	100
6G425	YKVSNRF	<b>EGVPDRF5</b> 1	SGSCTDFT	<u>LRISRVEAEDLO</u>	LYFCSQSTHVP	ltfgagt <b>klelk</b> r
		# 1		幸 材料的商品 难怪		# # #
F(ab)-1	YKVENRF	SGVPSRFS	SCSCTOFT	LTIBSLOPEDFA	TYYCSQSTHVP	LTFGQOTKVEIKR
2 (50) 2	AR ###		•		* ####	
humkl	YEGSTLE	SCVPSRFS	ssgsgt <b>dft</b>	LTISSLOPEDFA	<b>ТҮҮССЭН</b> ИЕҮР	Ltf00gtrveikr
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	L2				L3	

#### Variable Heavy Chain Domain

6G425	EIÖLÖÜSGASTUK ASVAAK TACAMAGARARSA HAMAKARURUS DEN T
	# ## ## ## # ### # # # # # # # # # # #
F(ab)-1	evolve600glyopgg5lrl8CAASgy5f55hymmvrqapgkglemv
	# ## # #
hvmIll	evolvesogolvopggslrl8CAAsgfsstghwmwvrQapgkglewv
	三元 2 3 3 8 8 8
	+44**
	<b>H1</b>
	50 a 70 80 abe 90 100



**FIG. 30A** 

IC50~12nM

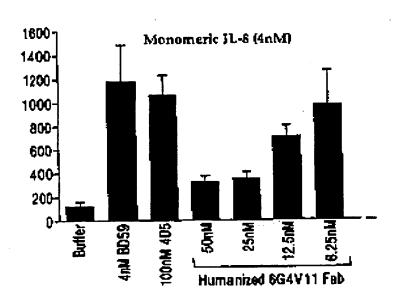
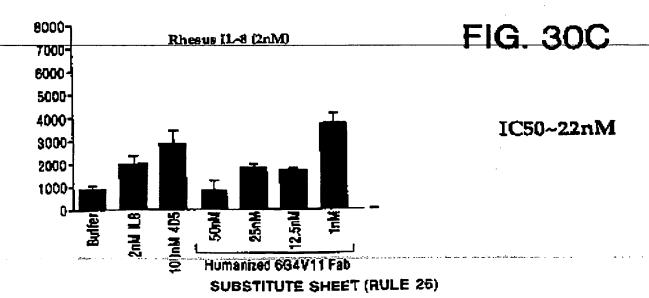


FIG. 30B

IC50~15nM



anti-IL-8 6G4.2.5V11 Light Chain Amino Acid Sequence of the humanized

ALQSGNSQESVITEQDSKDSTYSLSSTLTLSKADYEKHKVYACE\7THQGLSSPVTKSFNRG LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLMNFYPREAKVQWKVDN MKKNI AFTLASMFVFSI ATNAYADIQMTQSPSSLSASVGDRVTTTCRSSQSLVHGIGNTY

anti-IL-8 6G4.2.5V11 Heavy Chain Amino Acid Sequence of the bumanized

WVROAPGKGLEWVGYIDPSNGETTYNQKFKGRFTT,SRDNSKNTAYLQMNSLRAEDTAVYY CARGDYRYNGDWFFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK MKKNIAFLLASMFVFSIATNAYAEVQLVQSGGGLVQPGGSLRLSCAASGYSFSSHYMH **JUKKVEPKSCDKTHT**  Amino Acid Sequence of the peptide linker and M13 Phage Coat (gene-III)

SGGGSGSGDFDYEKMANANKGAMTENADENALQSDAKGKLDSVATDYGAAIDGFIGDVS GLANGNGATGDFAGSSNSQMAQVGDGDNSPLMNNFRQYL.PSLFQSVECRPFVFSAGKPY EFSIDCDKINDFRGVFAFILYVATFMYVFSTFANILRNKES

# FIG. 31A

1	A2	rga	A.	AA	GA	ATAT	CGC:	ATT	TOF	rett	rgca	TCI	ATG	TT¢	<b>5</b> (	rrrrr	TCI	'AT	TGC?	'AC	MAAC
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241																GTTC					
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	G	TAI	<u>~</u> A:	<b>GG</b> 3	CG	AGTY	<b>KACK</b>	ACC	TGT	ĆCC	atgg	<b>ጥ</b>	ÇÇA	CCT	CT.	AGTI	TGC	TTG	YCY	CCC	ACGT
58	H	7	V	P	L	T	F	G	Q	G	Ţ	K	V	E	I	K	R	T	V	A	A
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						GAC	TAG	ADAE	TG	CGG(	CTG	: G:	PAG	CACC	CGG	GAT	CATY	GCGI	TG	УTA	AGCAT
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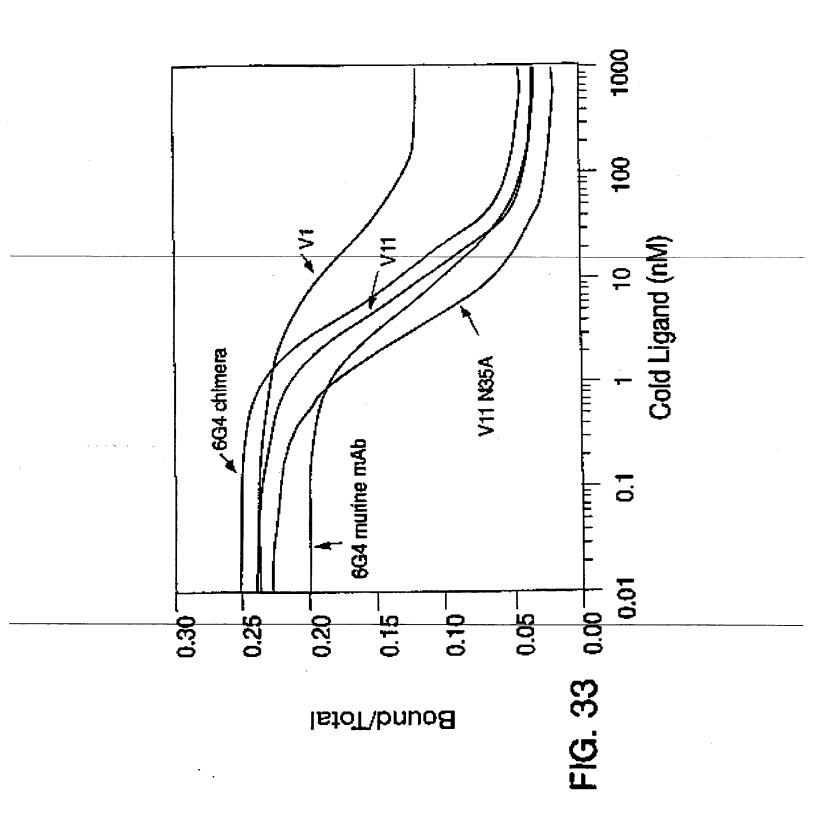
anti-IL-8 6G4.2.5V19 Light Chain Amino Acid Sequence of the humanized

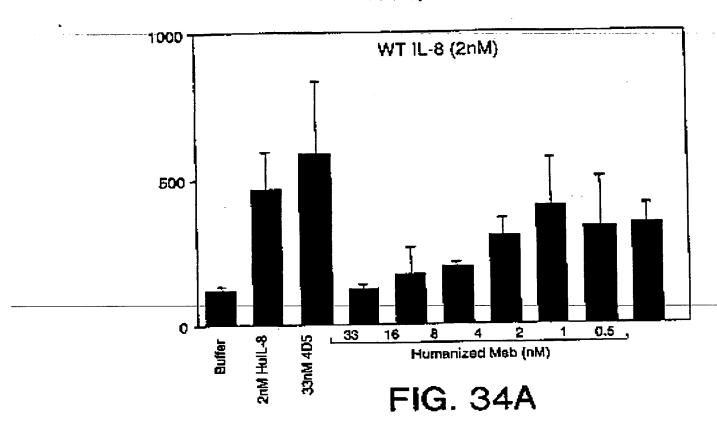
ALQSGNSQE\$VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG LHWYQQKPGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTLTIS\$LQPEDFATYYCSQST HVPLTFGQG¶KVETKRTVAAPSVFTFPPSDEQLKSGTASVVCLLMNFYPREAKVQWKVDN MKKNIAFLLASMFVFSIATMAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGNTY

anti-IL-8 6G4.2.5V19 Heavy Chain Amino Acid Sequence of the humanized

WYKQAPGKGILEWVGYI DPSNGETTYNQKFKGRFTLSRDNSKNTAYLOMNSLRAEDTAVYY PEPVTVSWN\$GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG†QTYICNVNHKPSNTK CARGDYRYN¢DWFFDVWCQGTLVTVSSASTKGPSVFPLAPSSKS†SGGTAALGCLVKDYF MKRNI AFLLASMFVFSIATNAYAEVQLVESGGGLVQPGGSLÆLS¢AASGYSFSSHYMH **VDKKVEPKS¢DKTHT** 







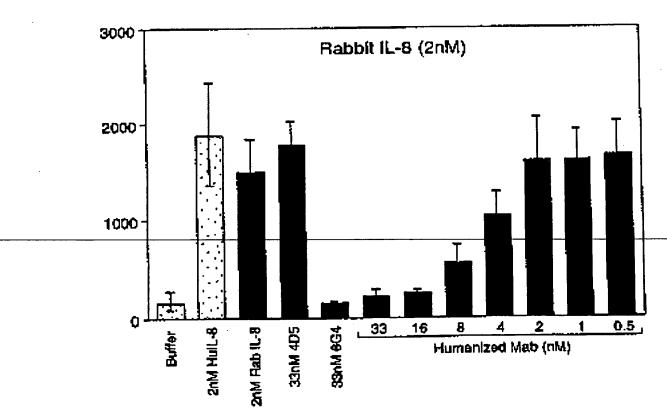
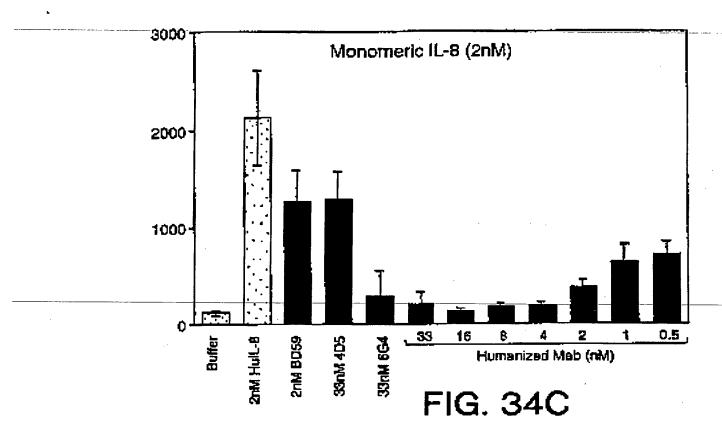
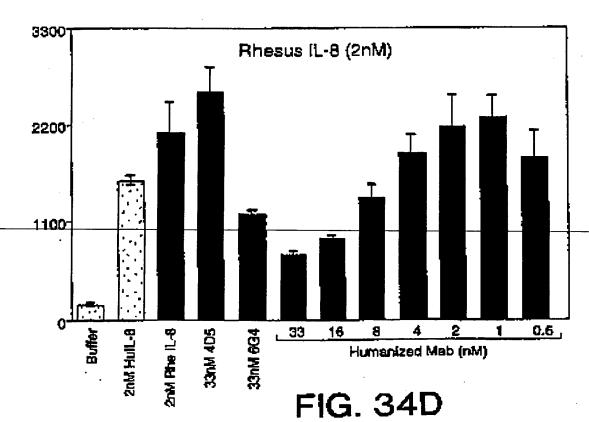


FIG. 34B





anti-IL-8 6G4.2 SV11N35A Light Chain Amino Acid Sequence of the humanized

ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG HVPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLMNFYPREAKVQWKVDN MKKNIAFILASMFVFSIATNAYADIQMTQSPSSLSASVGDRVTITCRSSQSLVHGIGATY LHWYQQKPGKAPKLL|IYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQST

anti-IL-8 6G4.2|5V11N35A Heavy Chain Amino Acid Sequence of the humanized

PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTK WYROAPGKELEWVGYIDPSNGETTYNOKFKGRFTLSRDNSKNTAYLOMNSLRAEDTAVYY CARGDYRYNGDWFFDVWGQGTLVTVSSASTRGPSVFPLAPSSKSTSGGTAALGCLVKDYF MKKNI AFILASMFVESIATNAYAEVQLVQSGGGLVQPGGSLRLSCAASGYSFSSHYMH **JOKKVEPKSCDKTHT**  Amino Acid Sequence of the putative Pepsin Cleavage Site and GCN4 Leucine Zipper

CPPCPAPELLGGRMKQLEDKVEELLSKNYHLENEVARLKKLVGER

1	atgaaaaaga					
					atabaaaaa	
-23	M K K N	I A F	L L A	8 M F V	F 6 I	ATN
61	GCATACGCTG					
_	CGTATGCGAC	TATAGGTCTA	CTGOGTCAGG	GGCTCGAGGG	DADDDDDADA	ACACCUGCTA
-3	A Y D	T D M	TUB	L P Z T	a w d	v G D
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7.0	K A T T		<del>19</del>	<u>. L </u>		<del></del>
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101					ATGACTARAT	
3.0	L H W Y					
	· · ·					
241	AATCGATTCT	CTGGAGTCCC	TTCTCGCTTC	TCTGGATCCG	GTTCTGGGAC	GGATTTCACT
					CAAGACCCTG	
58	NRFS	G V P	S R F	S G 6 G	S G T	DFT
301	CTGACCATCA					
					TAATGACAAG	
78	r T I S	s L Q	PED	F A T Y	A ¢ Z	OST
361	CATGTCCCGC					
					AGTTTGCTTG	
98	H V P L	<u>T</u> FG	Q G T	KAET	K R T	A A Y
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491						CCACCTATTG
139	V C L L					
200	, , – –			•		
541	GCCCTCCAAT	CGGGTAACTC	CCAGGAGAGT	GTCACAGAGC	AGGACAGCAA	GGACAGCACC
	CGGGAGGTTA	GCCCATTGAG	GGTCCTCTCA	CAGTGTCTCG	TOUTGTOGTT	CCTGTCGTGG
158	ALQS					
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601	TACAGCCTCA	GCAGCACCCT	GACOCTGAGC	AAAGCAGACT	ACGAGAAACA	CAAAGTCTAC
	ATOTOGGAGT	CCTCCTCCCA	CTCCCACTCC	TTTCGTCTGA	TGCTCTTTGT	GTTTCAGATG
178	ч э ь в	S T L	T L S	K A D Y	BKH	K A A
		•				
661	GCCTGCGAAG	TCACOCATCA	GGGCCTGAGC	TCGCCCGTCA	CAAAGAGCTT	CAACAGGGGA
						GTTGTCCCCT
198	A C E V	т н о	a L s	S P V T	K S F	n r c
		_				
721						ACTAGTCGTA
_		ADADDATIAD	TOUGGUETGE	GTAGCACCGG	GATCATGCGT	TGATCAGCAT
216	EÇO					

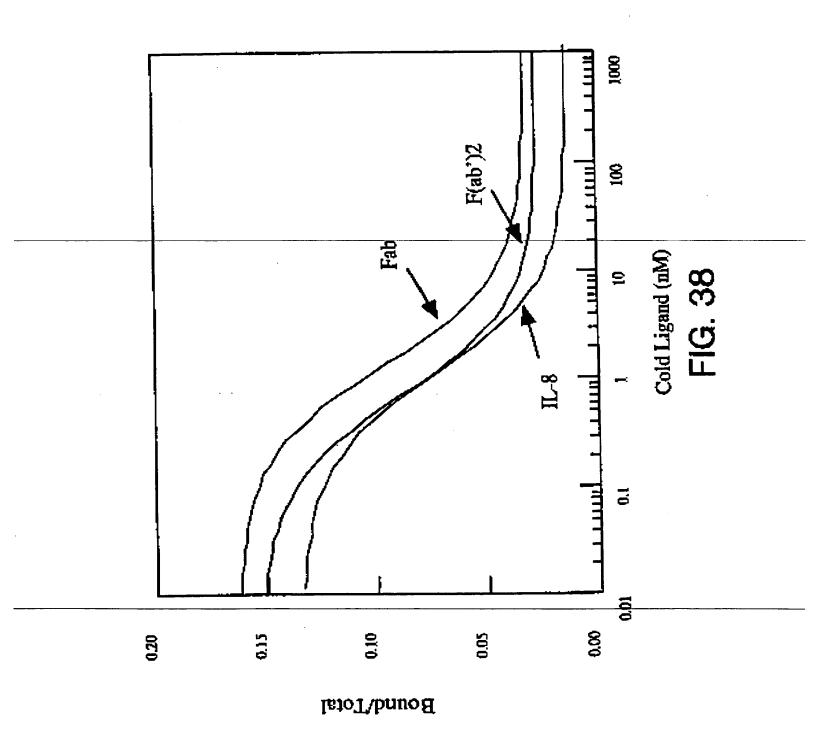
. 781	DAAAA OTTTT-	ATOO	T CTI	አ <mark>ው</mark> አርርር ኮርሞራር	TTG	AGG1	GAT	TTT	ATG ፕልሮ	AAA TTT	AAG! TTC:	. A!	Y <b>ra</b> t Vara	:GC)	TT.	TCTT AGA	TOT LAD!	rgca Acgi	k C
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961	TCCTT	CTCG	A GTO	CACTA	TAT	GCAC	TO	OTC	¢61	CAG			GGG/	AAT mma	366	CCT	GGA COD	SPTA	3
28	AGGAA	E	er Car	GIGA: H <u>Y</u>	M	EGIV	W	V	B.	Q	A :	5 F	G	ĸ	G	L	В	W	_
1021	GTTGG	TATAI	'A TT	GATC	CTTC	CAA	rgg:	AAD1	ACT	PACG	TAT.	A A	TCA	AAA	GTT	CAA	GGG	CCQ:	r
48	CAACC V G	ATATO Y	T AAI	CTAG	DAAE	N	G	E	TG.	T T	Y	N T	AGT	K	E.	K.	<u>G</u> .	R	P.
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1141	GCTG! CGAC!	OADO/	A CT	GCCG GCGC	ATOTA TADA	ATT TAA	CTG! GAC	TGCA ACGT	AGI	rece rece	XJAT CTA	T A A I	TCG AGC	ATO TAD	CAA GTT	TGG	TGA 'AC'I	CTG GAC	G C
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128	PS	٧	F	P L	A	P	В	8	K	S	Ŧ	S	G	G	T	Ä	A	L	
1321	GGCT	GCCTC	X3 TC	AAGG TTCC	ACTA TGAT	CTT	CCC .GG3	CGAA CTT	<b>0</b> 3/	TDD KOO	GACC CTGC	#G !	YIDI XADA	GTE Cae	AAE TYD:	CTY GAG	iac etc	3000 3000	*C
148	G C									v	T	V	S	W		.8			
1381	CTGA	CCAGO GGTCO	20 GC	GTGC	ACAC	CTT	GGG	GGCT ICCGA	GT CA	CCT: GGA!	aca( Yey	TE AS	CCT(	CACC	iact Tgr	CTI GAT	veda Laevi	CCC1 30GX	ic ve
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	L ACTC	TGTG/	TA CC	<b>GGCX</b>	PICAC	: GGC	TC	TOOT	CI	TGA	LCGA	CC	CGC	CGG	CCT	N CT	ŤTG	TCG	AT
221	т н	T.	C	P I	<b>,</b> ¢	₽	Å	Þ I 🏠	B	L	L	Ģ	G	R	М	ĸ	C	L	

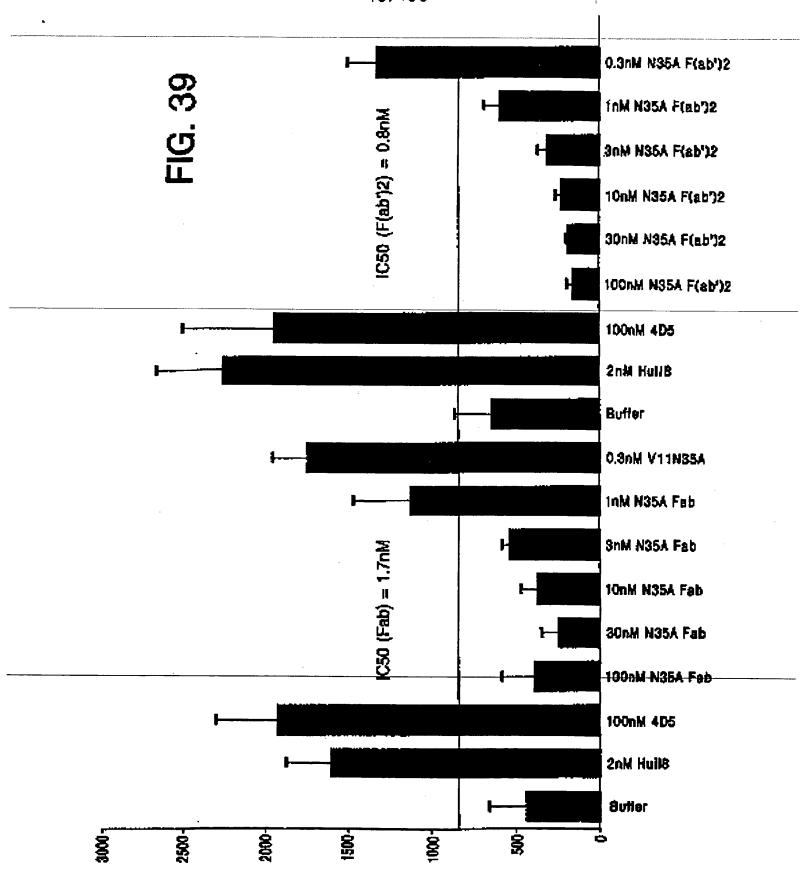
## FIG. 37A

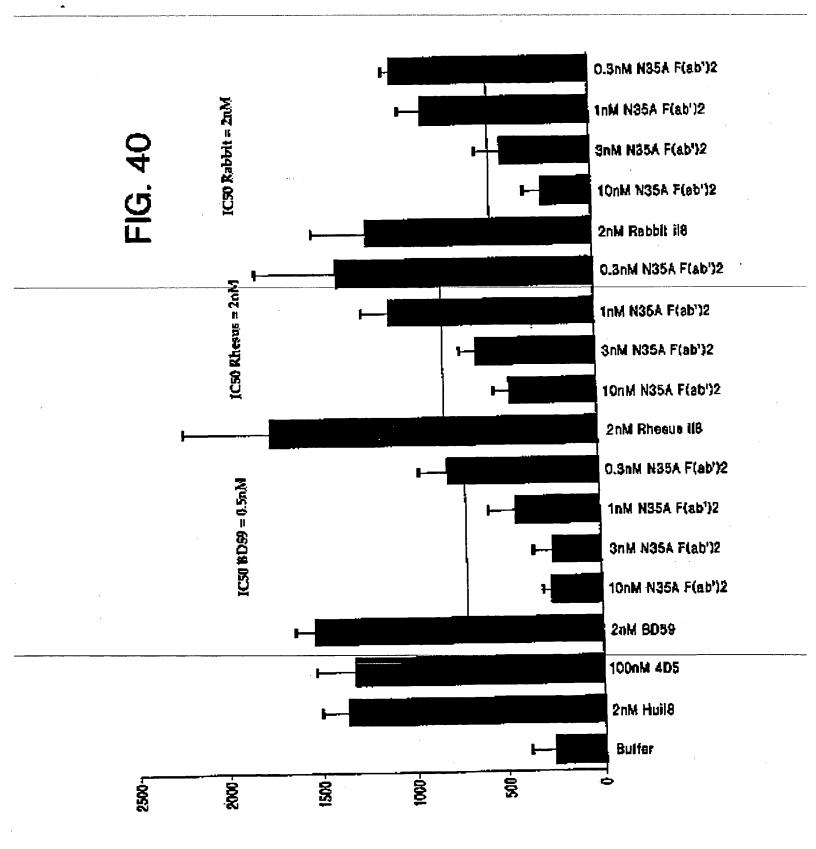
1621 GAGGACAAGG TCGAAGAGCT ACTCTCCAAG AACTACCACC TAGAGAATGA AGTGGCAAGA CTCCTGTTCC AGCTTCTCGA TGAGGGGTTC TTGATGGTGG ATCTCTTACT TCACCGTTCT  $248\ E\ D\ K\ V\ E\ E\ L\ L\ S\ K\ N\ Y\ H\ L\ E\ R\ E\ V\ A\ R$ 

1681 CTCAAAAAGC TTGTCGGGGA GCGCTAA GAGTTTTTCG AACAGCCCCT CGCGATT 268 L K K L V G E R O

FIG. 37B







SUBSTITUTE SHEET (RULE 26)

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	aeti nspbli accaacacc feerrence	aagaag <b>ita</b> Eitgitgaat	trosí mseí rutirarges paraferes	
alui bindili dael tro91 bsrDi msei cac91 rcarrccrca groffatty AdcTTGCCC	bírpi Bal/cfoi cccalabro cccalabro	thai frushi hushi hwai faushi haofi maeli havi maeli haofi haofi baohi baohi babi habi habi habi habi haofi haofi habi haofi h	1051 Grożeki igitifikate Castera acarantar	
nlaifí ba Reagac aighnasac Eca	maeli bardi Affancera ciechaich mestale Farbechet Gachiace	CG&CGATACG GCTGCTATGC	Hell/pall  if  if/xmalfi/cc  if  if  if  bradi/cc  bradi/cc  cccccctsa	
TROCALTARGG AMATACA		OROBI STATI DEST CHACGCA GCATTOCTGA CHACGGA CGATAGGACT	AGT.	
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FIG. 41A

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CAAAAAGAAI AJCGCATTIC TRCTTGCATC CTUTTTCTTA TAGGSTAAG SAGAAGGTAG E E N I A P L L A S E N I A P L L A S E N I A P L L A S E N I A P L L A S	hphi maeili saeili hpl setii hpl peterii hpl peterii hpl peterii hpl petericatae concactare compredate s
	benki beli avel 1111/aspi cccastococ ccostased
scrff bcil mapl hpali daav xmal/pspAl scrfl cauli bsal aval sau3al taqi cauli bsal abol/udci[dam-] csp61 dpul[dam-] csp61 dpul[dam-] hgici bsall alvi[dam-] hgici bsall alvi[dam-] asp716 bsall aval acc651 alvi[dam-] mali rcgcracccc cccracaca ccrccracacaca ccrccracacaca ccrccracacacac	ecory tth cor racenceater cor racentrace d 1 0 m T
scret  scret  scret  say  cauli  ban  aval  rsal  cauli  ban  aval  rsal  cauli  ban  aval  rsal  cauli  ban  aval  aval	SO1 ATACGCTGAT TANGCGACTA

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tf11  nf1 hemer  clat/bspin6 plei bsppi[dem-] hinfl  n rccarfcrcr gaacr	TACEGETCAC AIGACAAGEG F C S Q	CATETGANGA GTAGACTACT S D B
bi tagi AAGTATCOA	CGCAACTIAT GCCTTGAATA A T Y	acii mboii Arcinecee Tachaecee
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766h	921 AGC AGC A	
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etebi hpbi ancocatic	cacs muli cacs del didi gocrosse sergence cogacocace aciccicata i r a b r i		H 25 12
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beall deav  aval betwl beall bell  sau961 apy1[dcm+]  blaif  haefil/pall asu1  abu1 eccol091/draff haefil/pall  TCAGGCCCCG GGIAAGGCCC fGGAATGGGT  AGACGCCCG GGIAAGGCCC CATTCCCGC AGGTAACCCCA			macii hinli/acyi shali/besti gi aatii kacorc regesrcaas krecae acccrassrc b v w e e e e
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bsaJI aval bsaJI bau961 bae111/palI abul eccol091/dra ACTCCCCCG GGI ACTCCCCCG GGI	thai frontimor hatti hat		maeffi phi bsrf r Grencrecze CACTGACCA
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hacil/pell sau961 scri ccil ccil bpall caul dasy bell cfil01/barFl acil arccocrac record crecoccar	Fouthi 1266 acil basil/ HRAI BCTI easi FI bairi cfri GCACTOTCCG ACCCTTGG	mull sau3Al mbol/ndell[dem-] dpn[[dam+] dpn[[dam+] dpn[[dam+] dpn[[dam+] dpn[[dam+] dpn[[dam-] dpn[[dam-] dpn[[dam-] dpn[[dam-] dpn[[dam-] dpn[[dam-] dpn[[dam-] dpn[] hstul nlall hstul hstul hstul hstul hstul hstul alwigam= hstul hstul alwigam= hstul alwigam= hstul hstul alwigam= hstul alwi
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FIG. 41I

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	Pali Pali GGACTGITG CCTGACAG CATAGGGGG
hindi hint/ nlar/ nari kasi kasi byici bebi bebi sbaii/	ecrei  beli  pali  deal deav  beli cauli  saugéi haelli/pali  ball cauli  asuf beli cauli  eccl091/drall  eccl091/drall  ecclcccc ccccccc cccccc  corcccc ccccccc cccccc  corcccc ccccccc cccccccccc
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his his plant part part part part part part part par	CITCITACGG GACAAAGCC CCCAACCTA GGACTGGAT
	2701 1980

FIG. 41J

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for the form of th	Bau96I  Diary  Avbil  Bail  Bail  Acti  TGCACGCCCT COCTCAAGCC TYCGTCACG GTCCCGCCCCCCCCCCCCCCCCCCCCCCCCCC	meri eagl/maiii/eclii eagl/maiii/eclii eagl/maiii/eclii eagl/maiii/eclii eagl/maiii/eclii eagl hippi eagl thai thai cfii hhaifeld thai fuubii/mani cfiil/bexri i hpaii haori habillasi cáchi acii hgai bgti nlaii haciii/peli macii cach cgccgccnnc ccccccnccnc ccccccnc cccccccc
GTAGGACAG		MACIX CRCBI
	tfil binfl TYCGGRATCT AAGCCTTAGA	alli/eclii hibri thal/cfol thal fuubli/mvni brtul brtul (CC CCCCGCTA
mboli bpual bbsi praterene concental	Pall am-] csc81 cscreccc11 ccaacsca1	ncri eagl/pmaill/eclii eagl/pmaill/eclii cfri hhal/cfol cfri hhal/cfol nael fou491 fuuDII/mvn cfri01/bsxFI brt01 chell haoFI brt01 chell acii hgel hgil alail haelil/peli xgccgGCXIG GCGCCCGG
	n haelii/pali saulki mbol/kdeli(dam-) dpul[dam+) dpul[dam-) cs ka tcsccortic cc	
acii fuugui bsoZi carcectock	thai foubli/evol betti bebiti6: s hibbi hai/cfol d geoi[dcm-] d GC GCGACGATG	haelll/pol flaer forceratat
	thai foubli/evoi bethi behi3361 sau hibby mbb hhai/cfoi dpi bpei/geoi[dcm-] dpi reccrocace ccerecateca	haeIII/pe:   haeI   pacitive:   cardi   cardi   coccutive:   coccutive:   coccutive:
acil thai thai fuvbil/wui bstül alalii hshi2361 acii hshi260i halfcioi halfcioi halfcioi bsog	acil sen961 avell asel rccroscer	meeli pepi406i Chaccerro
2960 1060 1060	# COOK	370 C

FIG. 41K

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D=WFI alui alwi[dam-] gagatgaoca ccarcagosa Cagctricas archaggost ggragroch grogaagfic	11 byl286 byl286 belgKAI bmyl csccoacc acardcaacc Gerreccafc GAGCCCTCC TGRACTICC CCACCTAC	palí fouffi beofí acii malí hpalí nialv baei bgàci tagi cfriol/beifi ccalí bait rcancrdaa rocaadcrd cocadcros
bapWit faubii/mvni scrfi faubii/mvni mvai batui mvai mvai batui mvai batui acali daav cccommaca sccumicci rcasscasc geccanaca sccumicci rcasscasc	sau961  avaii sau3ai  sau3ai atui sbol/bdeii[dam-]  abol/bdeii[dam-]  apoi[dam-] apoii apoii[dam+]  apoii(dam-) acii apoii[dam+]  captacres accersare dreacsca	hatil/pall saus61 saus61 stri that that not1/mvol tepl foud11/mvol hetor betor daav betor betor betor asul teq beh12361 beh12361 mlary asul malr molf act hyaf act nlary asul malr molf act accorded coeccent forces
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FIG. 41L

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ther styl il bsaji iaa occtigadas sty gegaaccete	eau3AI mboi/odeif[dam-] nla idam-] idam-] ave pial/aspBI ave pilal/aspBI ave pilal/aspBI ave rpl286 ppuv pi beingal cyI moli cyI beingal togatese creencer	maeli GCA AAACSTCTGC CGT TYTGCAGGCG
hibPi hhai/cfoi usti pfiler avil/fepi bewi beli i ACEGEGATG OGCHARCCA O	The policy of th	bbyl foutti bbyl bcch ccchc recreticch
acii CTTGCGGAGA ACHGTG GAACGCCTCT FGACAC	haell/pall hael bael scrif sval daal ecoRll dsav bstMI bslf baell apyl[dcm+] ava, I hiapl dp ava, I hiapl dp ava, I mstf nlal) nlary cft avi ccolo91/drall msl gcarcrece cacsorec	thal hphi fnubli/rui batti  i hah1236i maaii rcacccanac acaaccaac acroscrac cacroscrac cacaaccac
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tru91  msei bpel/gsul[dcm-]  ACC CACACTERS COCTICTOCA GRANCTCCAACCTICG CTTCAACTERS	fnu4fil thai baori fnuDil/mvni alui batui pruli hinb! nuppli hhai/cfoi fnu4fi thai baori fnuDil/mvni begi batui bbyl mali bah1236i acii bbyl bah1236i hphi cccca cctcccccc cccrarccci dateaccac	bgal thal thal fowDil/wwni batut acti bah12361 biap! napBil hal/cfol hhal/cfol cher researanc coerteres
cac81  sau961  tru91  msel asul  acif bdir nlaiff acil  colabance decerrance reconsonance colorative consonance colorative critical and anotherse	xmul xmul xmul tfil tfil txin asp700 ms11 alul acii asp700 ns11 alul acii hcy	scrri beli mapl hpali sfawin foki dsav maelit achi cauri drdi ccorchchcc rrencina geschoods geschance
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FIG. 410

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	moli CCTC	CCATARCGCA GGAB	
bstll071 tru91 bsoE act acci bsx1 mest acti Accounts Alacrocal ActalGCGG	mboli eari/hsp6321 sapi hinPi hbal/cfoi heeli acii AGCCGTCTT CCGCTT	tfii bibfi Cagaateage Gaettageee	acit coscosita 41P
	efani acii a atacgegaic te tatggegere	ra cectatica at eccataest	
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•401 CGGGTGTGGG	ETA 1054	fo ac fourth backl bbv1 4601 GCTGC	4701 AX 111

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mepi (HI hpali fi beshi G CTACCGAT	hgial/asp bapi286 bsimkai bayi apali/sbo alu! apali/sbo alu! Alviti/sb	alwn[dcm-] fuu4HI bsofi fuu4HI bsoff hhvi mae bsri hhvi hsri ACTGGCAGCA GCCACTGGTA TGACCGTACAT	had. braccestic graccestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercestercest
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mspl hpaif sau3al abol/ndeif[dam-] dpai[dam-] aluf alwi[dam-] acii hgarcgacc refrance Gearacance	sau3Al sau3Al sau3Al sau3Al sau3Al mbol/bdell[dam-] [[dem-] dpoll[dam-] dpoll[dam-] shwl[dam-] shwl[dam-] shwl[dam-] chyl/kholl chyddahac gcanarc cccaacac carafac	eli(dem-)  tro9:	genial  mbol/odell[dam-]  dpol[dam+]  dpol[dam+]  accolcider Clarres Carceras foctors  rectagaca caraansea Grassarea accorcida
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haeffi/peli aau961 himg; asuI hhal/cfol AGGGCGAAC TCCCGGCTCG TCCCGGCTCG  matipi matipi matipi aviii/fspi	r TGCGCAACGI a ACCCGTGCA  eau3Ai mbol/odeII(dam-) dpoli(dam-) dpoli(dam-) trairi nlairi c AFGATCCGC G TACFAGGGGG
nspl hpell bgll caclectoca car cockococa car cockococa tru91	FACTOCCEA GYTAATAGTI IGGCCAGGI ATGAGGGG CAATAATCAA ACCCCTTGCA GAUSAI mbol/ndeli[dam-] mbol/ndeli[dam-] dpol[[dam-]
G CASTABAC GTAATATC	G TAGTTCGCCA C ATCAGCGGI  BOOL/U  GPUI[  GPUI[  CAACCACGA
bpel/gsul[dcm-] :: :: :: :: :: :: :: :: :: :: :: :: ::	AC CTACACTAB TC CATCTCATT DIALY MSPI DAANI ALUI DPAII AC CTCCGCITC
bpel/gs mspi hpaii cfri01/bsrFi hph nlaly rC ACCGCTCCA G aG TCCCCAAGGI C aG TCCCCAAGGI C ag tCCCCAAGGI C cauii bpaii r cauii blui	A ACCECCENT  G CTTCATTC  C CAACTAAGU
AI  I  MADI	TRITAATIG: ATBATTAAC FITTGCTATG
beal that that functi/mvol betoT bebill361 ecti artacceccac accc rarecceccac accc rareccecc	CCARCEGIC GGTAGGTCAC ELI ACGCTCGTCC TGCGAGCAG
fradei baori I berbi bbvi ccaccraare ccaccraac	TAMPONICA GG TAMPO
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68 / 136

5901 ANCTHORGCA AAAAACCEGT TAGCICCITC GOTCCICCEA ICCTIGICAG AAGIAAGIIG GCOOCAGTGI IAICACICAT GOTTAUGECA GCACTECAIA TACAACACGT TITHCCCCA AICGAGGARS CCAGAGGGCT AGCACAGA AIAGIGAGAA CCAATACCGI CSICACCAT

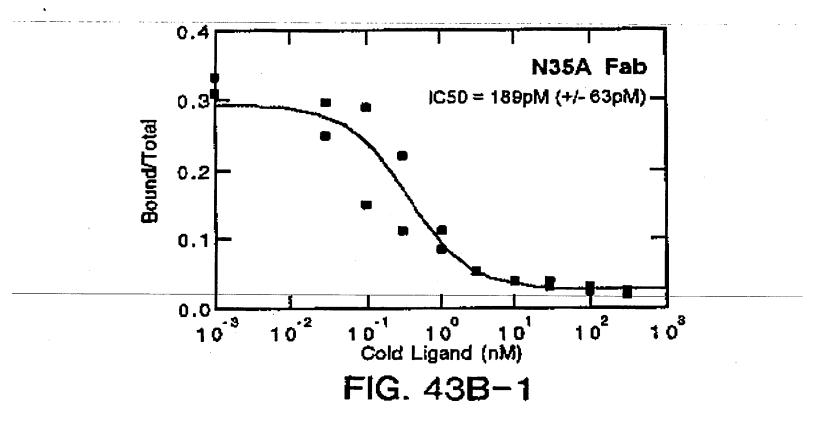
•			
	- 697)36 E		
GAGTTGCTC	saq3Al mbol/adell[dam-] dpol[dam+] dpoll[dam-] strl/xboll alwr[dam-] crcaaggarc crcaaggarc	GCHARACAG CSTTTTGTC	AFCAGGGFFA FACTCXCLAAF
mofi besti besti acti acti acti acti acti acti acti	COCCAAACT	hebi TTCTGGGIGA AAGACCACT	TGBAGCATH
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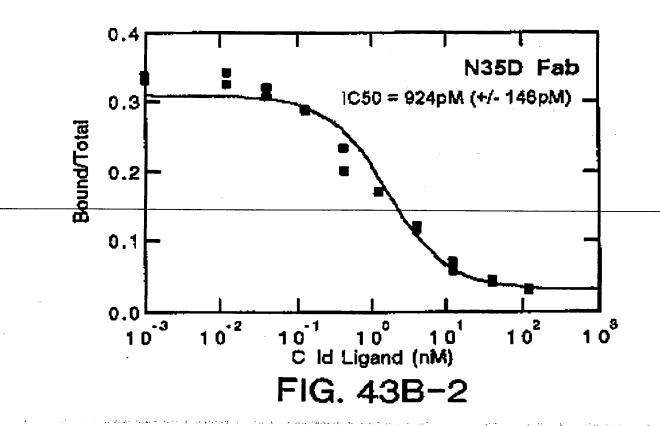
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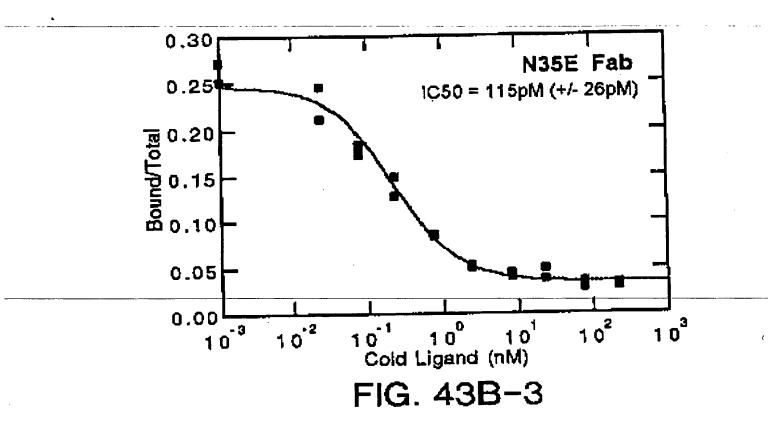
## FIG. 41V

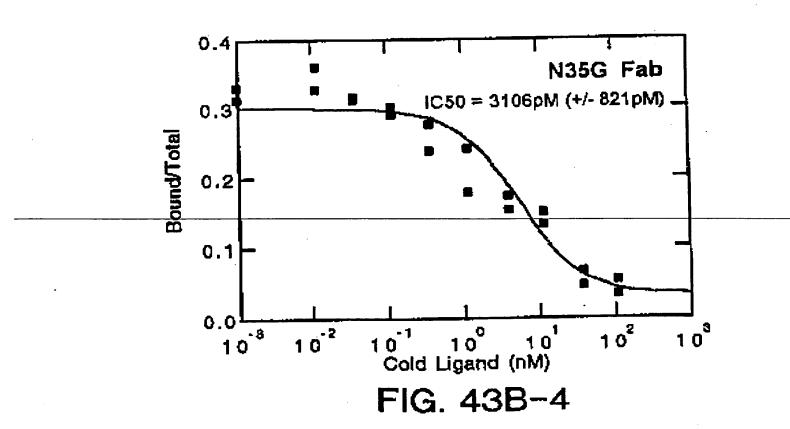
		r tat tta cac 3'	r tat tta cac 3'	
	Stop Template Primer	5' CAT GGT ATA GGT TAA ACT TAT TTA CAC 3'	NNS Randomization Primer SL.97.3 5' CAT GGT ATA GGT NNS ACT TAT TTA CAC 3'	FIG. 42
· · · · ·	Stop Tem	SL.97.2	NNS Ran SL.97.3	

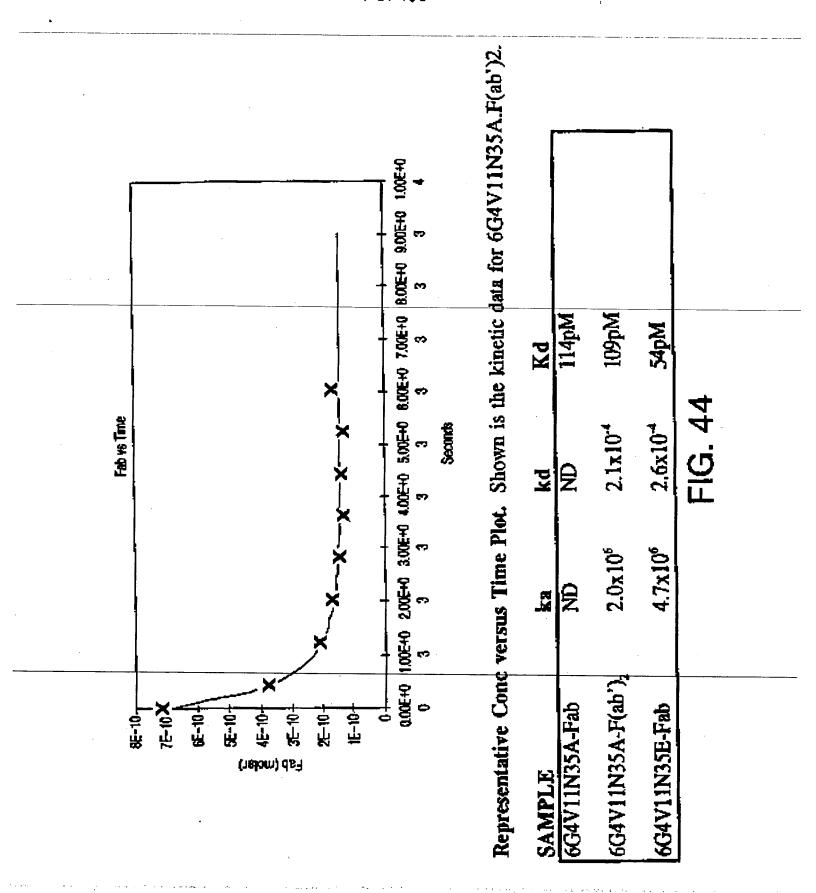
Am	ino Acid I	Amino Acid Frequency	Amino Acid Frequency
Phage Display (NNS Codon Library) Sort #3	(NNS Co	don Libra	ry) Sort #3
Amino Acid Fi	Frequency % Total	% Total	IC50 (nM)
Asparagine (wt)	$\leftarrow$	5.6	4.9
Glycine	9	16.6	3.1
Aspartic Acid	33	16.6	3.1
Glutamic Acid	4	22.2	0.1
Alanine	2	5.6	0.2
Lysine	<b>-</b>	5.6	ND
Serine	<del>,</del>	1.9	R







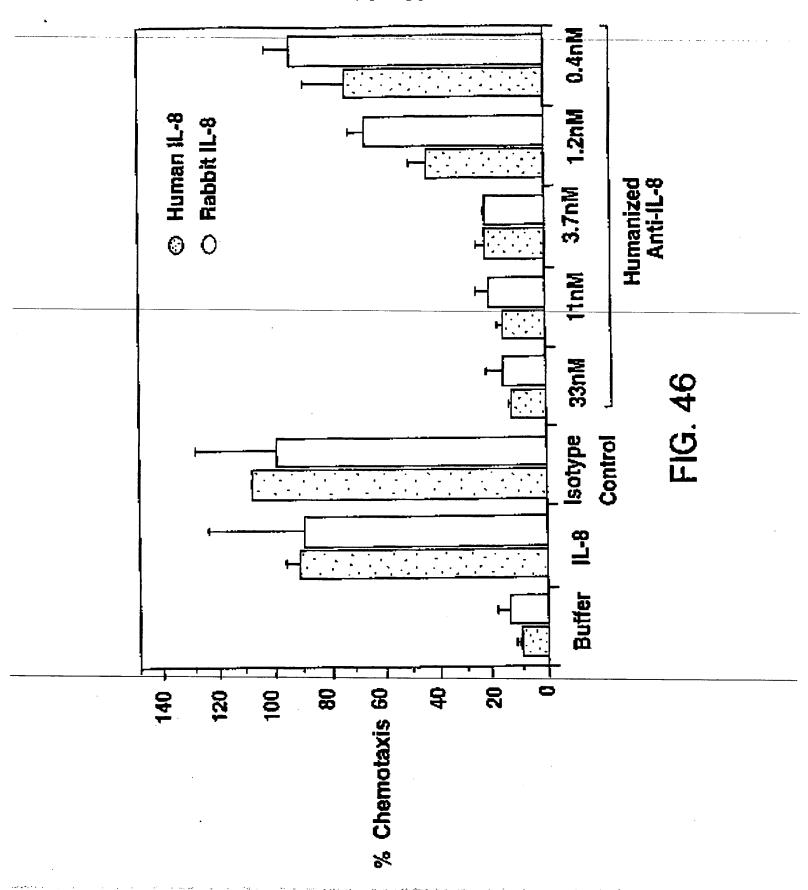




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2	16	E		C	Q																					

FIG. 45

78 / 136



79/136 S-CTAGTGCAGTCTGGCGGTGGCTGGTGCAGCCAGGGGGTCACTCCGTTTGTCCTGCGAGCTTCTGGCTACTCCTTC-3 5-TCGAGAAGGAGTAGCCAGAAGCTGCACAGGACAAACGGAGTGAGCCCCCTGGGTGCACCAGGCCACCGCCACGGCCAGACTGCACT Bold indicates nucleotide change destroying Pvull site. N35AH1upr N35AH11MT AG-3

SUBETITUTE SHEET (RULE 26)

	CARBI CCAGGGGA GGTGSTCCGR	PLUTCANTTA	goigachai tiittithat Coactgaita abhabatra
		ppul01 nsil/avaIII nsil/avaIII styl nsil/avaIII styl nsil/avaIII nool	bell deal cccccccccccccccccccccccccccccccccccc
100 (EE)			
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) end the introm o the Hpel Wite	GAATSIGTEF CAGITAGGST CITACAGAGA GTGAATGGGA	rai ecomii eav grmi heari beari olarv G recteaseer co	acil barl acil c caccadatra est s cuessicase est
aker (psv17 ksr(Lt) int	seu3Al alul mboi/ndeli dam.] dpu[dba+} vul/bepCl dpul[dam-) taql dam-) ori pvull sli bepBil sql(dam-) xarc cACAGCTGTS o	scrfl srai 60811  I deav betki npyl[den dem+] benbi alaiv Grerdeaan ructcadecr	CCCTANCTC GGGGNTTGAG
ict25v11.N35A.chcSD the pRK7 clouing linker (pBVI7) and the intro a linearization linker(LL) into the Bpal site	seu3Al alu nbol/ndell  dpol/des+} pvul/bepcl plel dpol/[dax-] hinf: taqi dax-] il belk: pvul Achdrostr chadd	SCIFI MTM  EVAI GOORIS  SCORII GOOV  GROV DETRI  DETRI  DETRI  SOLII D	acli foki certece getregee
	seu3Al alul  mboi/ndeli dam-]  dpu[[dem+]  pru]/DepCl  plel dpul[dam-]  thof: taqi dam-]  mae! balu! ospBl!  bfa! taqi[dam-]  attMTTGACI ACAGCTGTC GAATGIGTST CAGITAGGGT  AAATAACAGA TOTCAACTAG CTOTCAACACA GTCAATCCCA	attage	ecli GCCTARCTCC GC GCCATTCAGG CG
7 pp/tm.p6G oc with to adding a			151 171 171 171 171 171 171 171 171 171
7 18:27:36 1997  my/vc/lnmblo/sfs  mlift (circular)  he pSYI hackboo	PPET CCCARCATTG	APGCTTTCCC	BENET DEMET PATAGRAGAGE PATAGRAGAGE
> wed May 7 18:27:36 1997 > /home/ruby/vc/lombio/efmb/em.p6 > sitem: std > length: 8120 (circular) > This has the pSYI hackbook with > made from pSYI.WIRD.D by adding	cechi alui esti madi hgivii hgivii beliffi beliffi beni taqi trocaccito crcacatte	gaagtatgea Cttcataggs	acii bempi Greachage arachedese eceni Cagnesings ratuasedese eceni
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FIG.

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ruel Macî etyl	beath blb: avri[dam-] beali/peli tul asi 11 bfai GGCC TAGGCTTTTG	DOLL AGAGGAPTT TCT COTABAN	heelli/pall hael scrii sval eookil daay batki acil apyl(dos+) seli anli ddei corge corgectes
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·	hac moli 301 Tarccadagg	enrFI ncil mepi bpali damy cauli equecessaces	tagj SD1 TCACCATE ARCTGGTAAC

FIG. 48B

tfll truff ddel boll tegl abell/drel rerecaffra	tru91 afl1/bfr1 sfaM h&e1 GATGCCTAA CACTTATIGA	Dae(II/pali hael  Dval  COORII  deav  alii batmi ddel plei  binfi apyldcm+) binfi ATCARICAAC CAGGGRACA TACTERNYG GYCDGRICAA ATCYCAAAA	
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ecos  oboli  earl//s  indi  caagaara concaacat ff	trugi mpel seel/abni/rspi aggrengar fartatager rechercha attarantab	HPPI HPRII BRANT ACAROCHERA TICHCARA	
0 <b>5</b> 0 5	701 4 E	BOI &	

hgal hloll/acyX ehall/bakli scifi nval noll ebokli dsav bstni ecoNi apyl[dcm+] noll apyl[dcm+] noll apyl[dcm+] beliddel haarrahaac Crercocaca Aracccacac Grecocaca	noli alui care ferseresee teeraaasee caas asassassas assattessa	tru91 meel asel/aedl/yegi attatataca tancettate tattatacac taattatst attssaafac ataspatst	
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the tile seu 3 seu 5 seu	n+]    efall   example of AT		
thelli seusai abol/ndell[dan-] dpnl[den-] dpnl[den-] dpnll[den-] GTGACAAGGA ICAIGGAGGA ATITGA	BCUFI  nval  deav  bet#t  bpy[[dcm+]  sau95;  aval!  ssul moll  asciccacca scanashacc atcaactata	nla styl ocol ppuldi deal ocil/avalli bead Arcchitir Alagaccan	
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ds v tagl apol beth! tagl apol beth! tagl apol beth! tagl apol clal/baplo6 beal! bepDK[dar-] bebli bepDK[dar-] croccassic chartecast research generalized generation persection research.	scrFI sval fpu481 scrMil dasv bstWi bscPI spy[[dcs+] hael bbv1 acil haelil/pall rengeGene cerescan achecene cerescan scheene cerescan	perfit  peril  peril  daav  couli  xwel/pephi  easi  cauli  cauli  beav  cauli  beal  beal  beal  beal  heall/pell  beatl/pell  souff  coccoccocra  coccoccocra  coccoccocra  coccoccocca  coccocca  daav  vol  beal  beal  coccoccocca  coccoccocca  coccocca  daav  vol  beal  beal  beal  coccoccocca  coccoccocca  daav  coccoccocca  coccoccocca  daav  coccoccocca  daav  coccoccocca  daav  coccoccocca  daav  coccoccocca  coccoccocca  daav  coccoccocca  daav  coccoccocca  coccoccocca  daav  coccocca  daav  coccoccocca  daav  coccoccocca  daav  coccoccocca  daav  coccoccocca  daav  coccoccocca  daav  coccocca  daav  coccoccocca  daav  coccocca  daav  coccocca  daav  coccoccocca  daav  coccocca  daav  cocca  cocca  daav  cocca  cocca  cocca  cocca  cocca  cocca  cocc
ds v betNI apyl(dcm+) mnli c) baaJi beaJi pi CTCCCAGGTC CAACTGCAGC TCGGTTCTA GEGGGTCCAG GTTGACGTGG AGCCAAGATI	real recipions of the state of	BECTEL  BECTEL  BEAUT  BANDEL  BEAUT  BANDEL  BEAUT  BEAUT
ds v tagl apol hell henli clal/baplo6 hphi ecfi fok! bell baal beal beal bebli contenda treaters continta soremance area from parketitam	rsel bpel/gaul(dom-] berl cep6t Ancredaet Genetacatr Caen TYGACGITGA CENCATGIAN GICTI	plet  htpft  tagt  khol  pecs71  gecs71  kwel maelit  kwe
i Ingelancas Ccacittece R Atciaticia Gigmangg a	resi psel I foki besi Atcatoctif Tigiagiago Angtaggala Abgricatog	alui alwnider-) foudei bsofi bbwi TGTCTSTGC AGCTTCTGGC ACAGGACACG TCGAAGACCG S C n n S G
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FIG. 48J

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FIG. 48L

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FIG. 48H

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FIG. 48U

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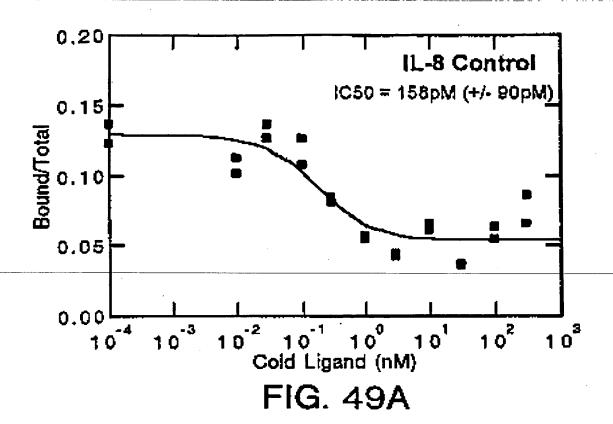
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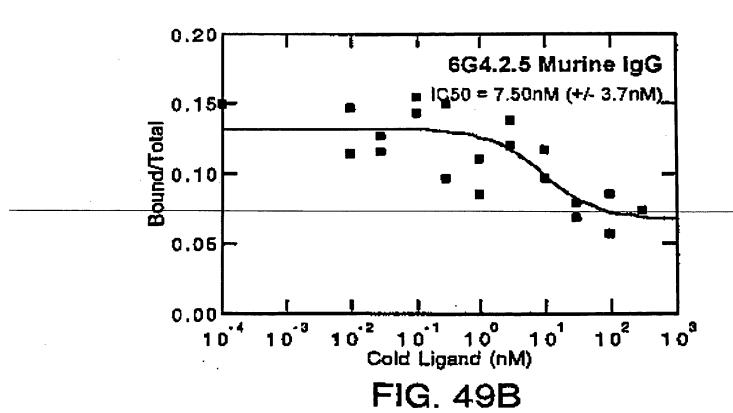
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fol GSCTTCDGGA GCGAAGGSCT	Tiatageet Pataterect	TITTACGT I	cec bsr81 ii eqii chranraccec
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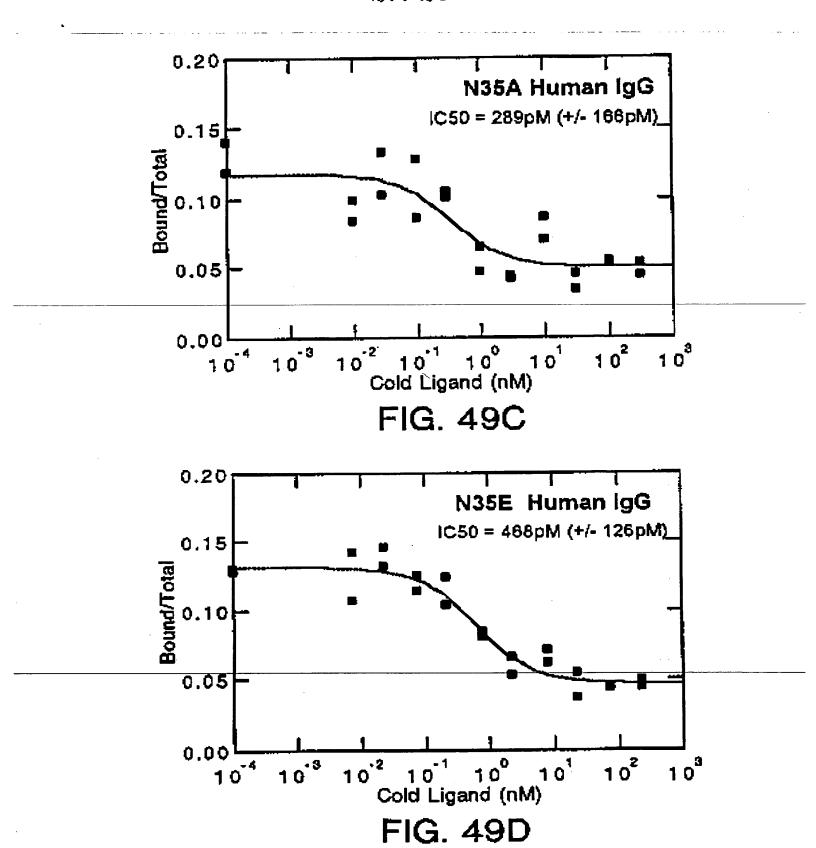
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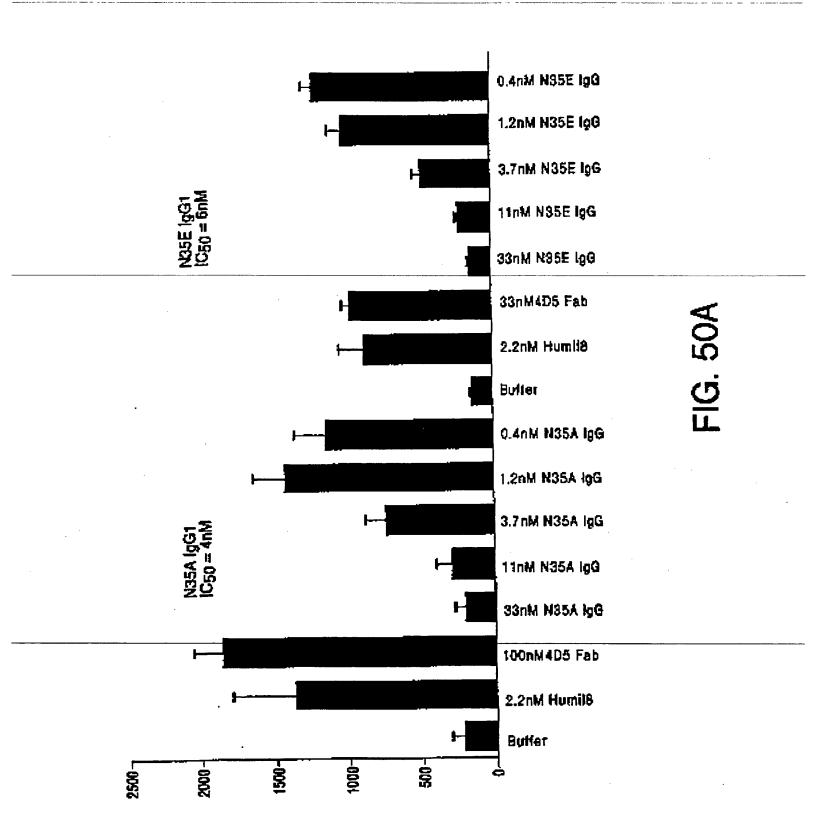
FIG. 48Y

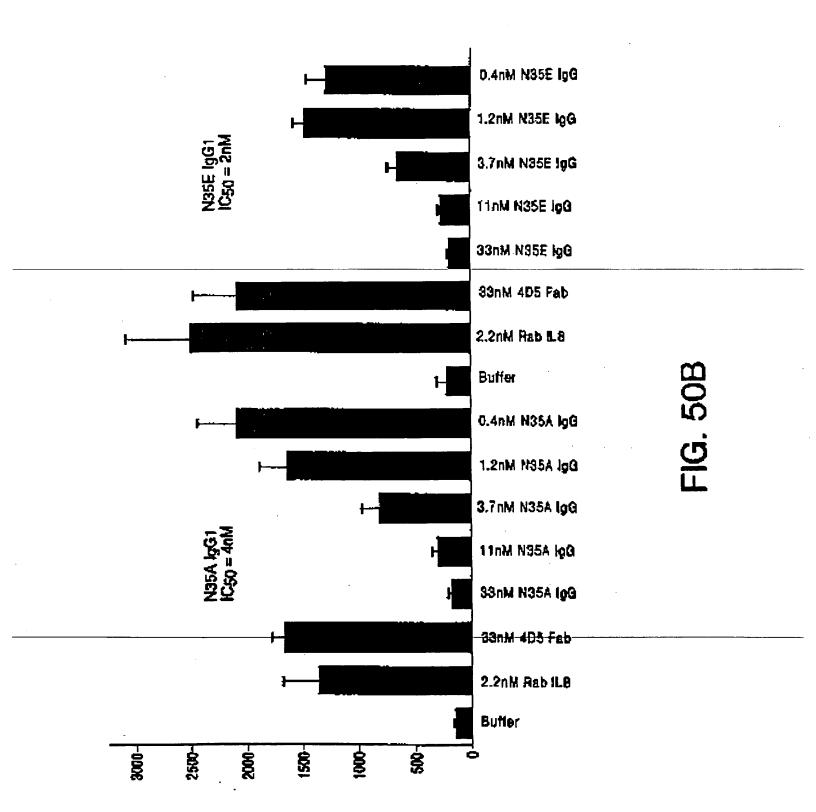
SUBSTITUTE SHEET (RULE 26)

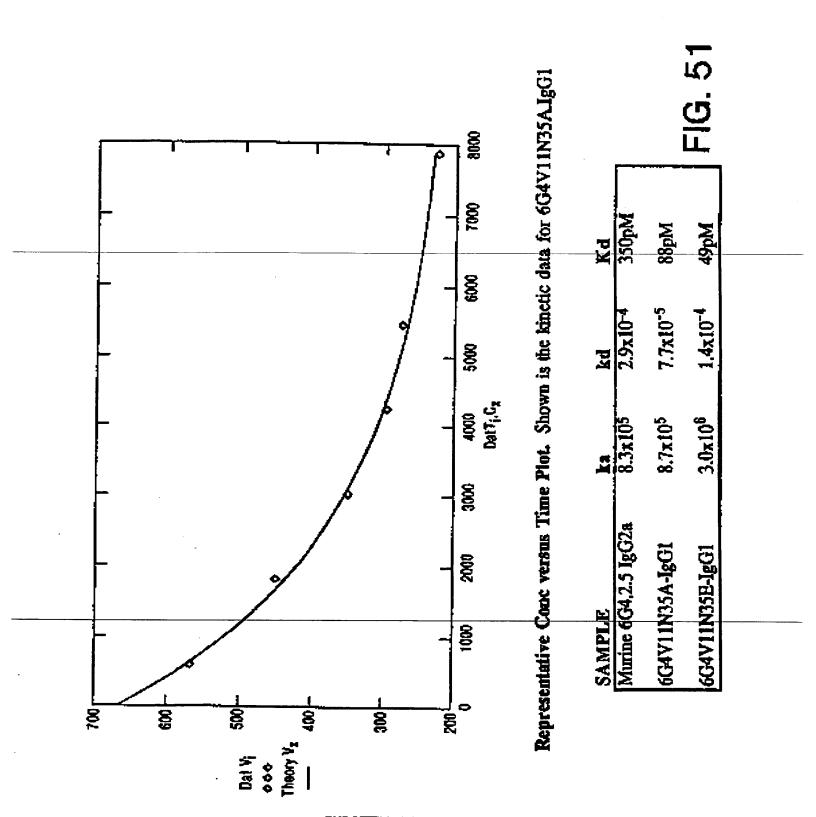




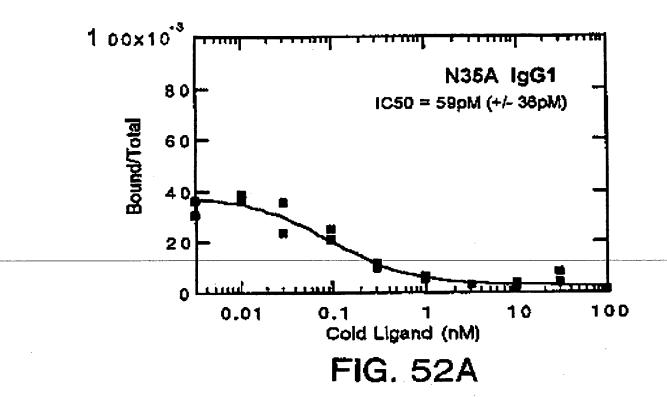


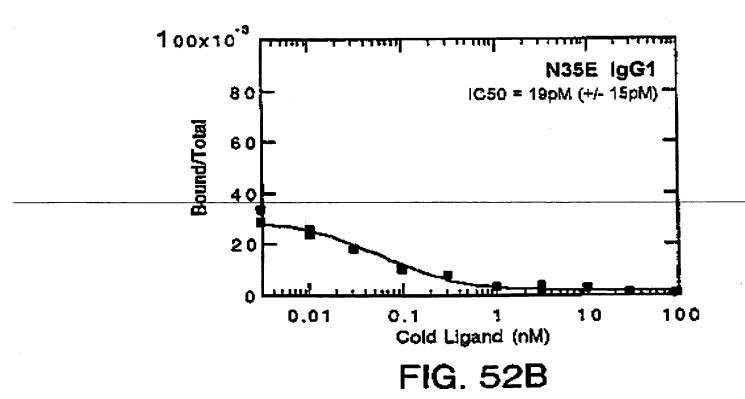






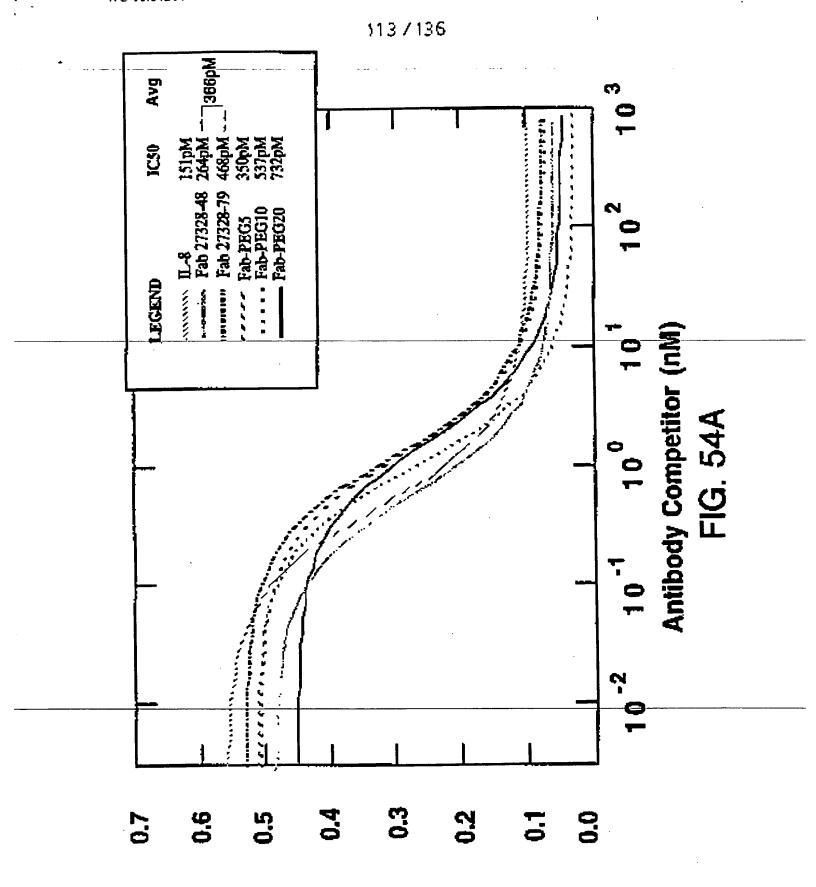
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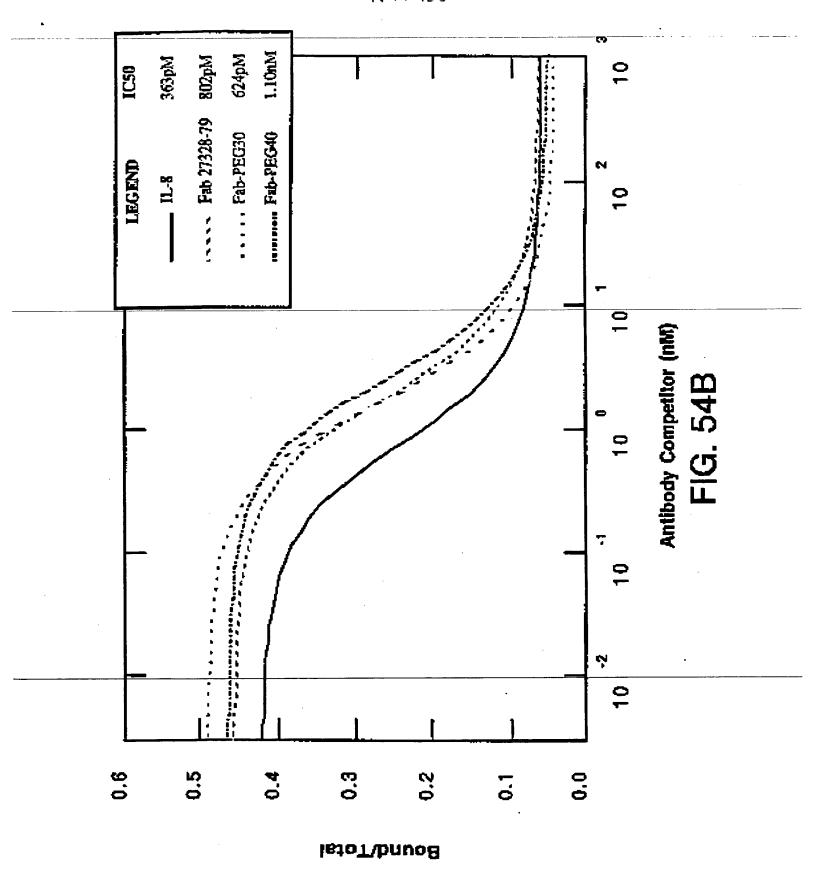


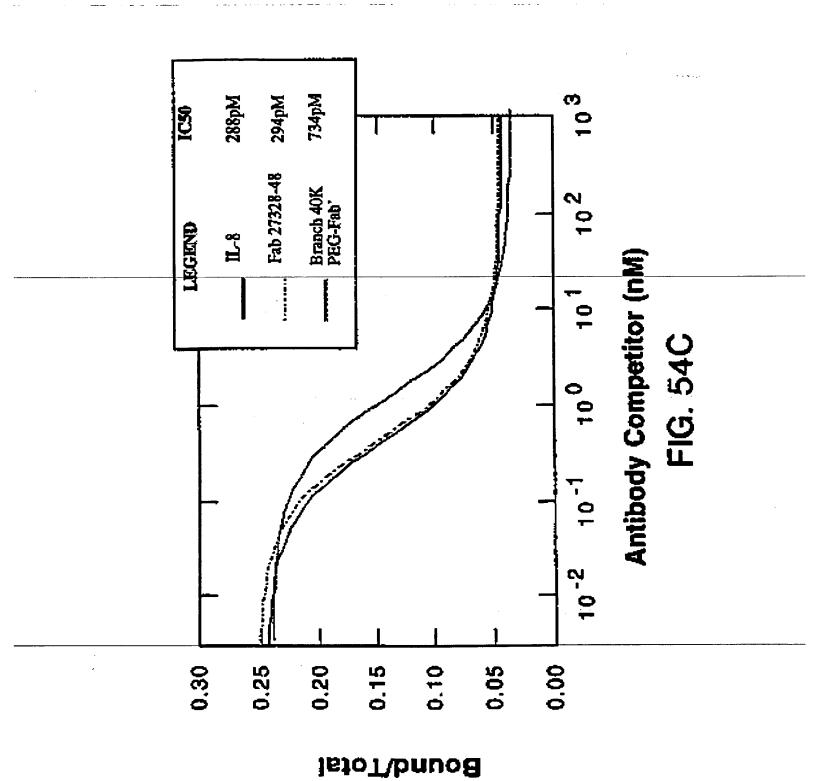
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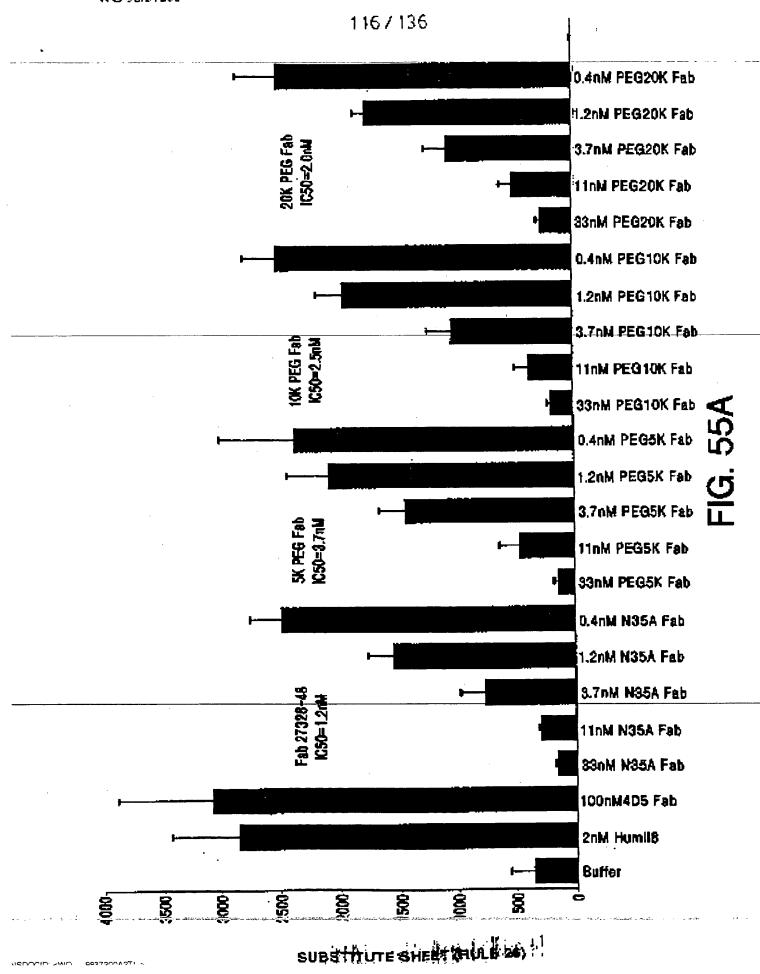


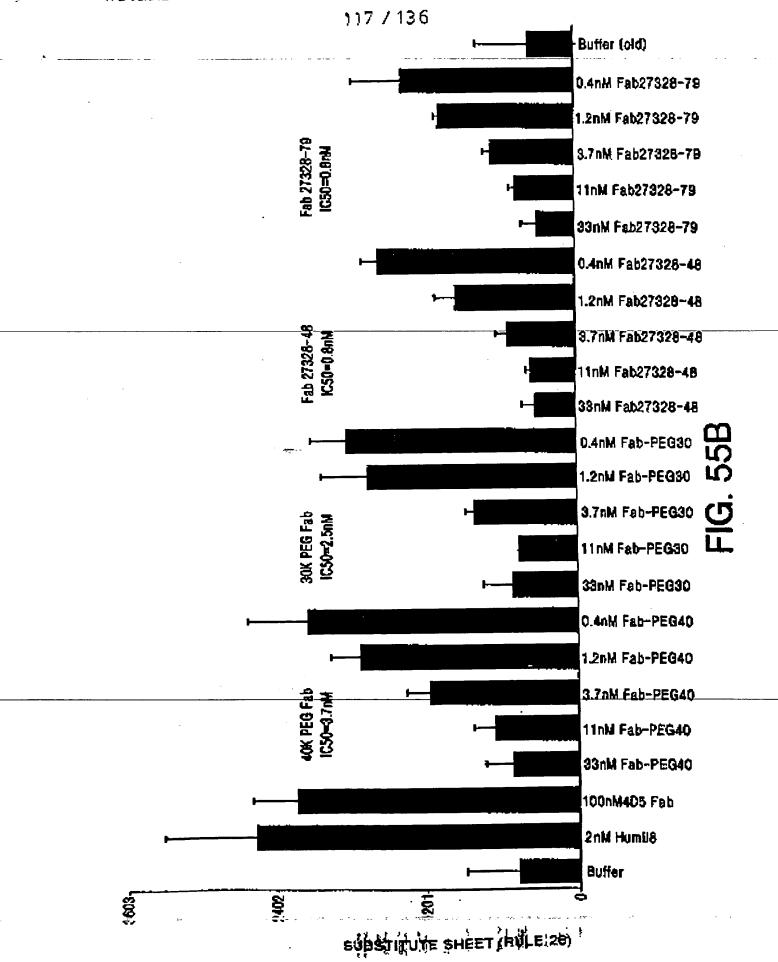
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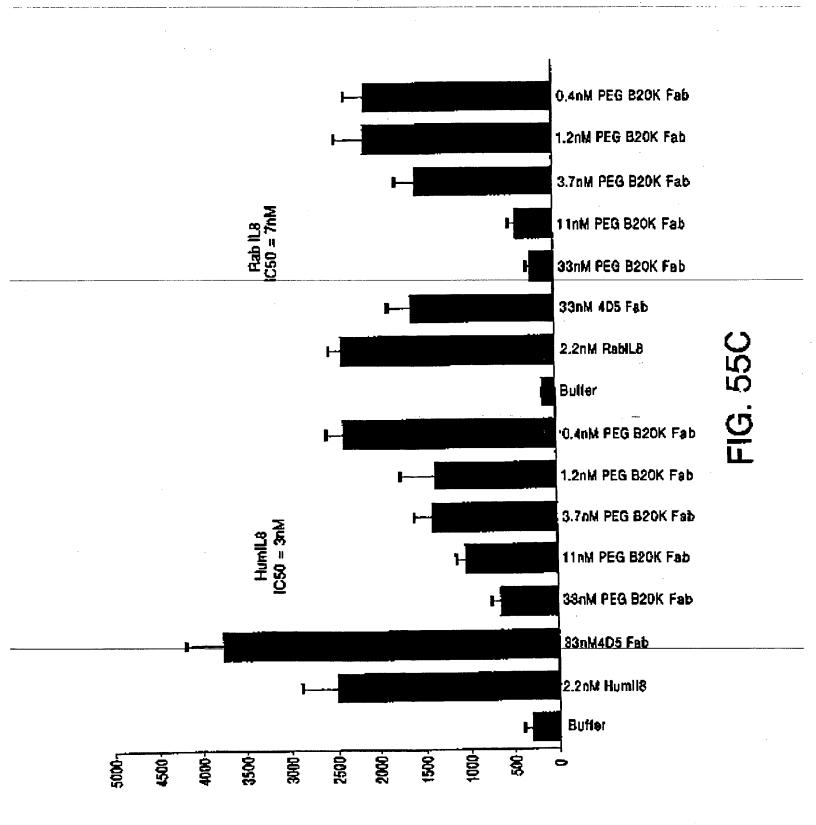


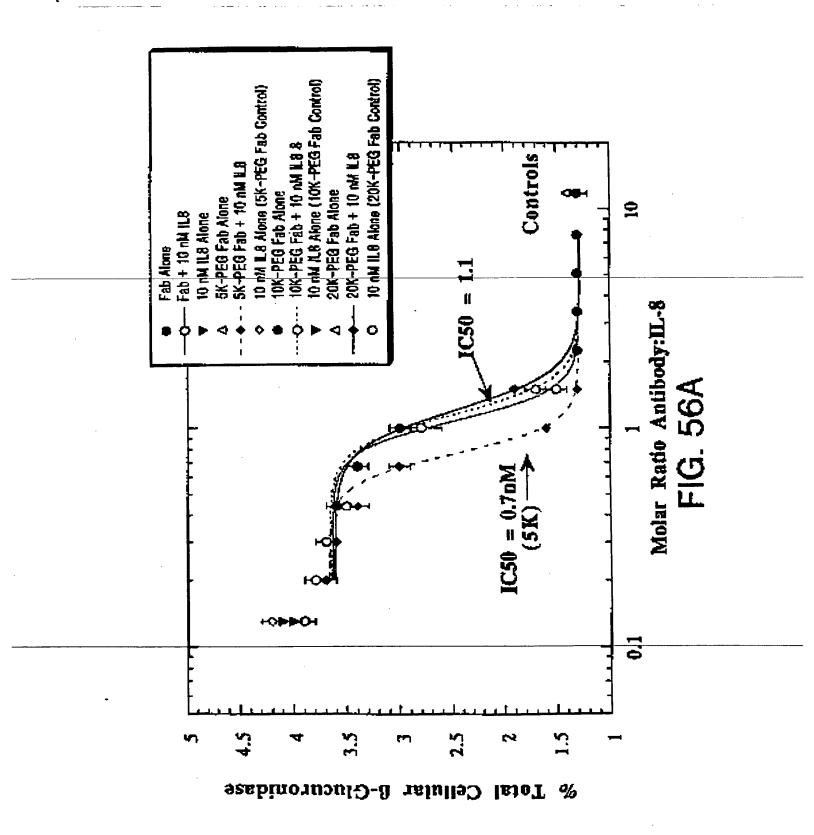


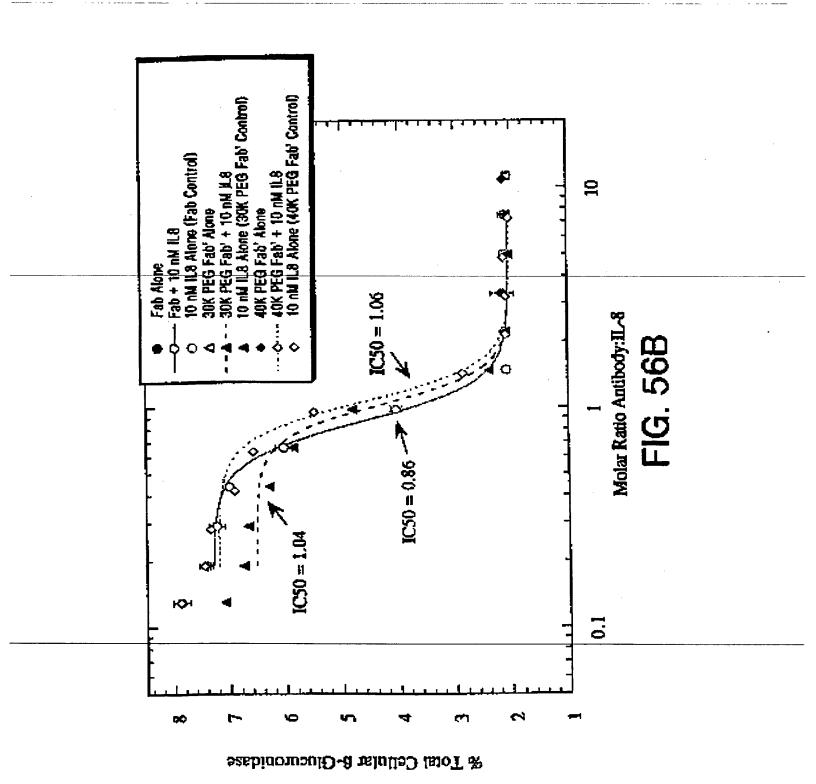
SUBSTITUTE SHEET (RULE 25)

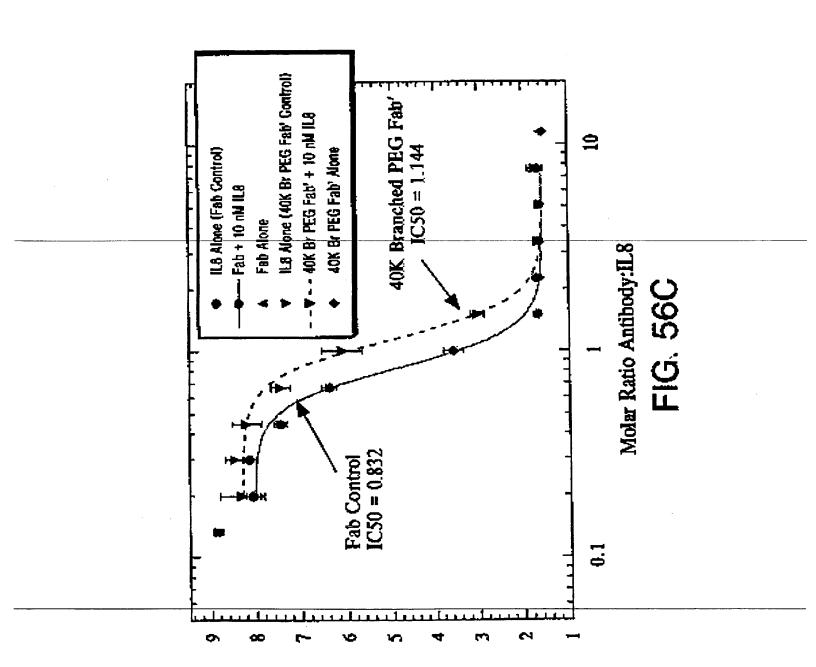




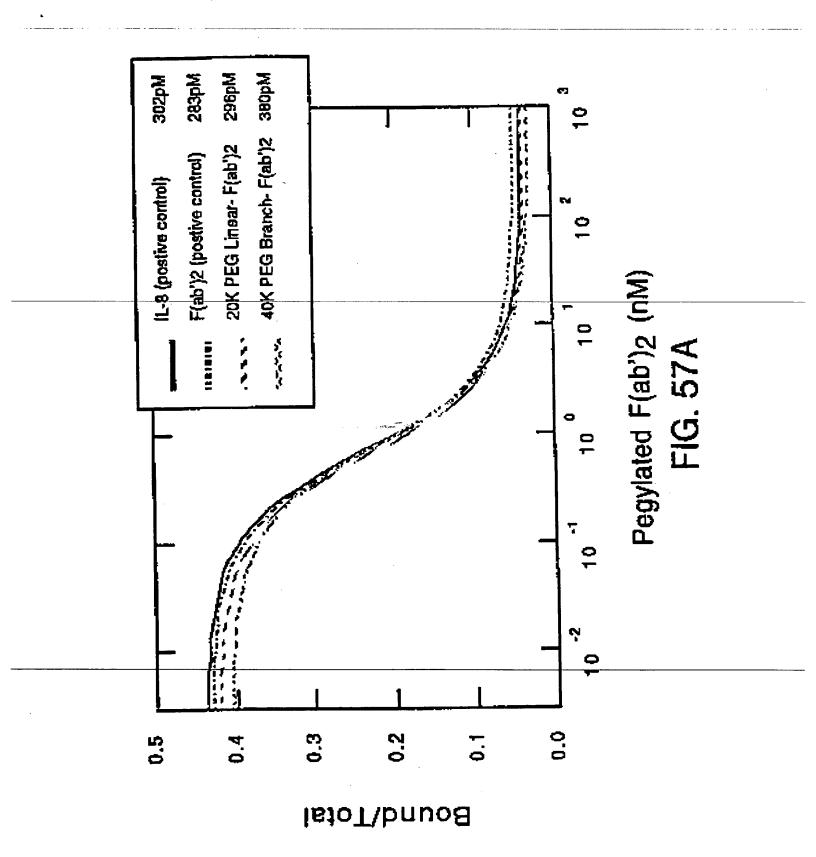


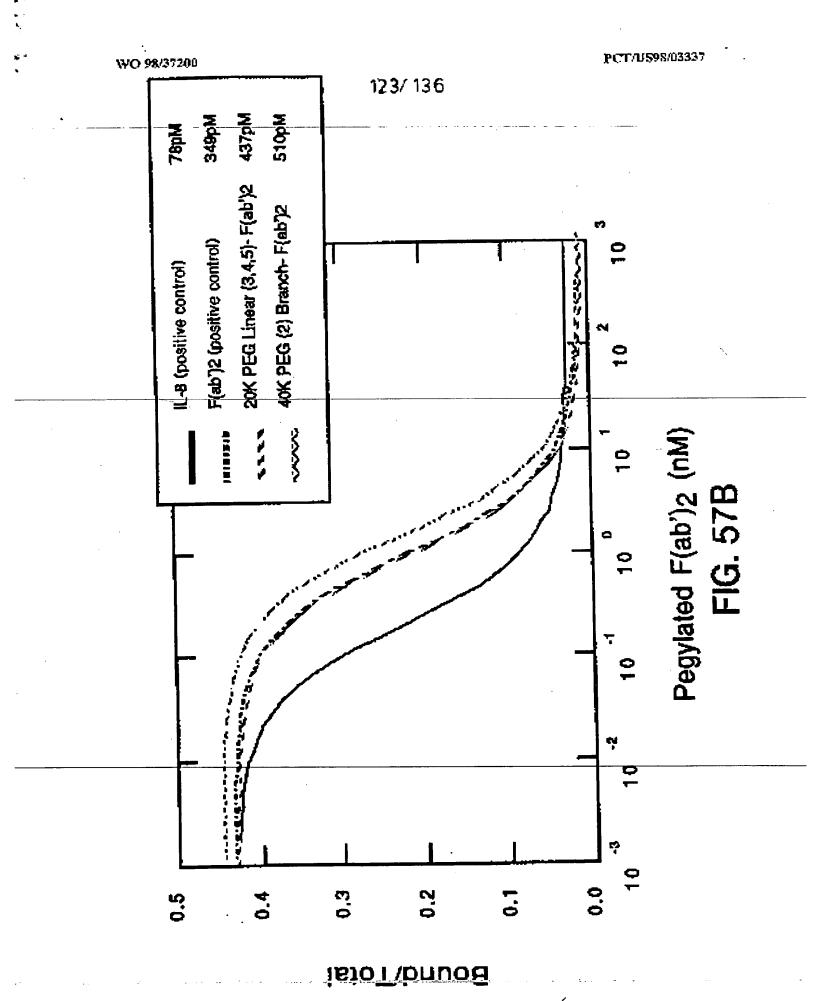




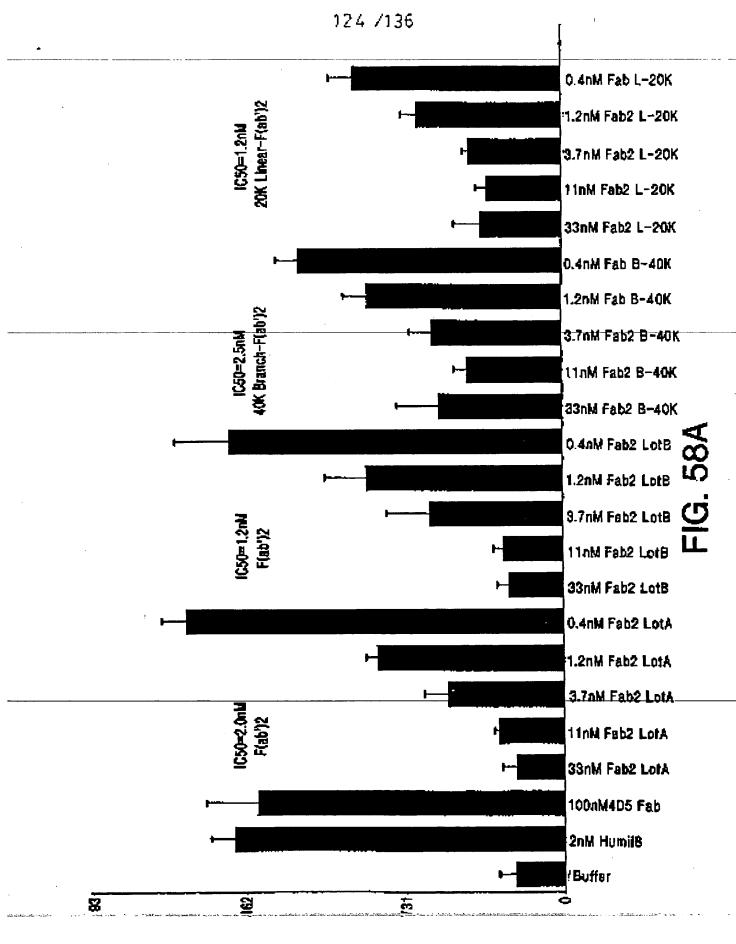


% Total Cellular B-Glucuronidase Activity

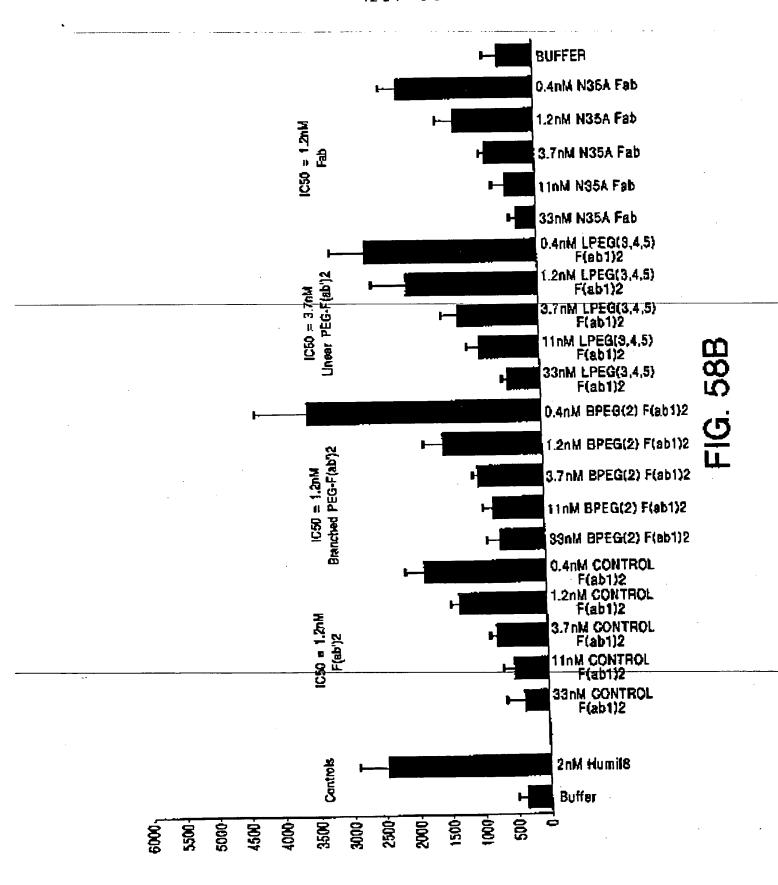


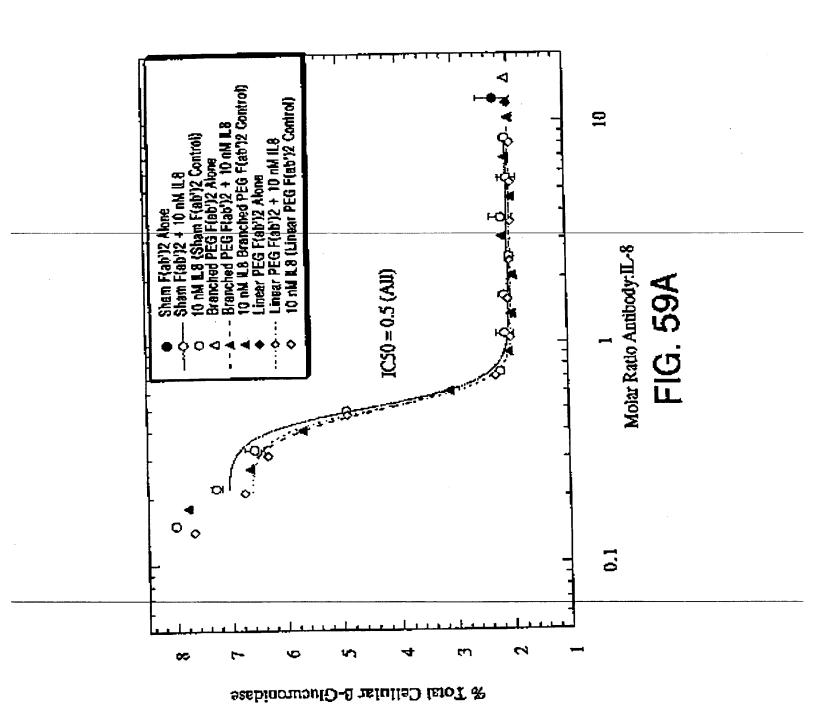


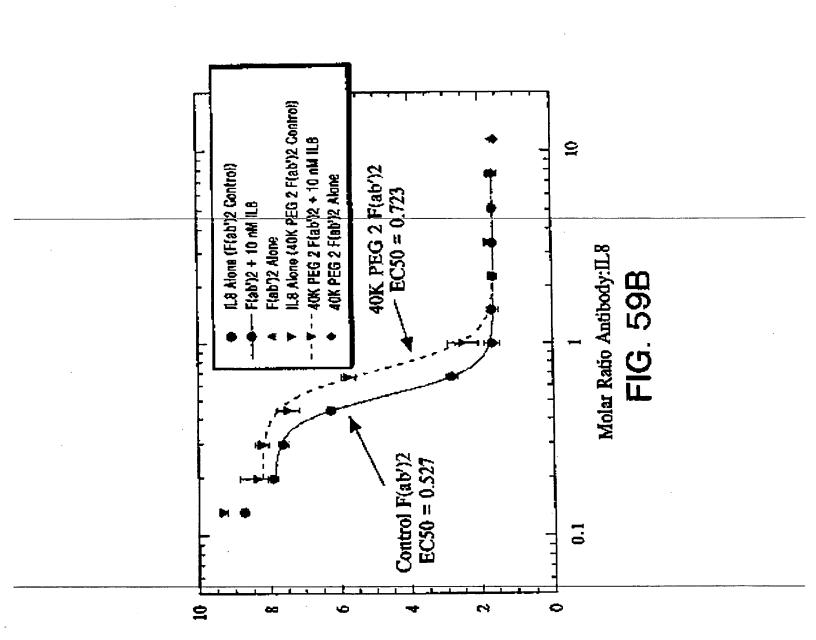
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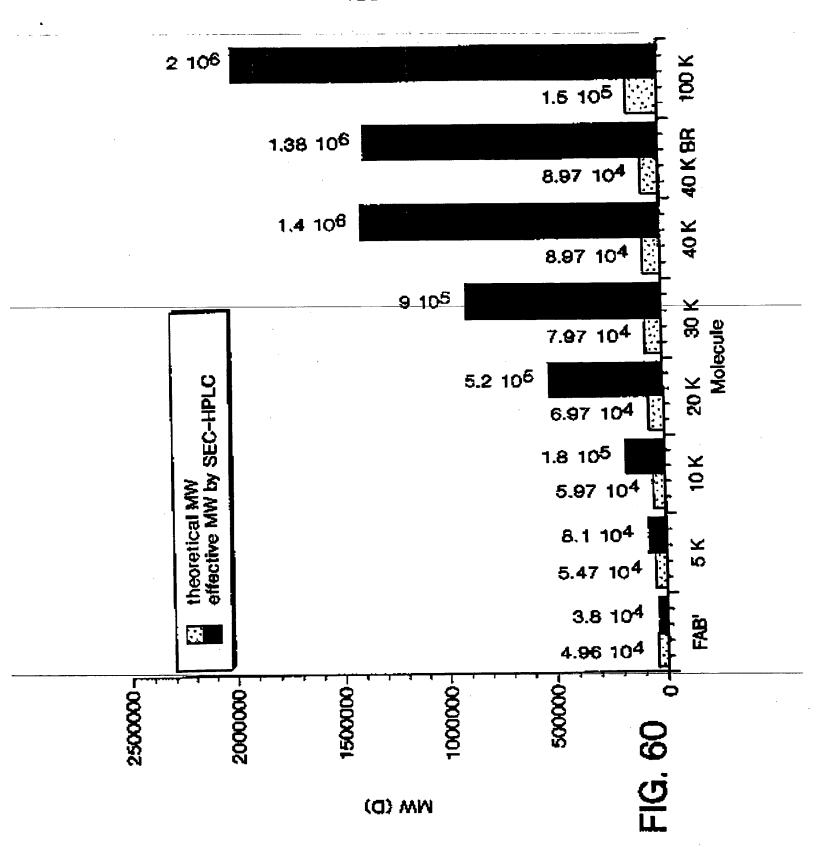






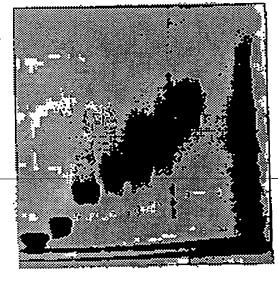
% Total Cellular B-Glucuronidase Activity

128/136



-5K -10K -20K -30K -40K -40K branch -100K

Reduced

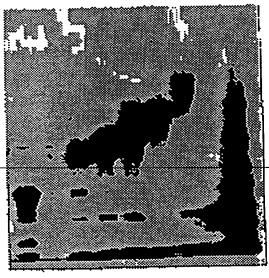


-220 G -130 G -100 G

-6© -40

FIG. 61A

Non-Feduced



-X20 G--X20 G--130 G--100 G-

-100 -70 -60 -40 -30 -20

FIG. 61B

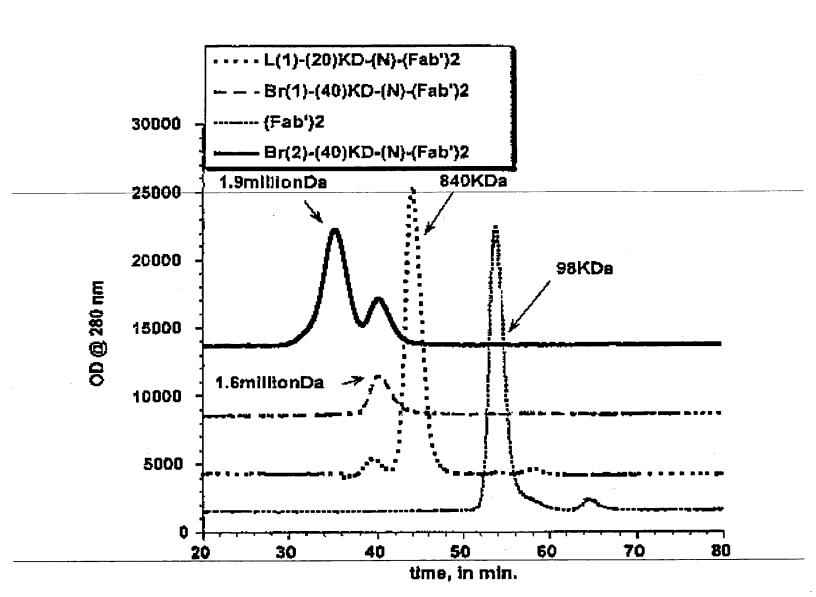


FIG. 62

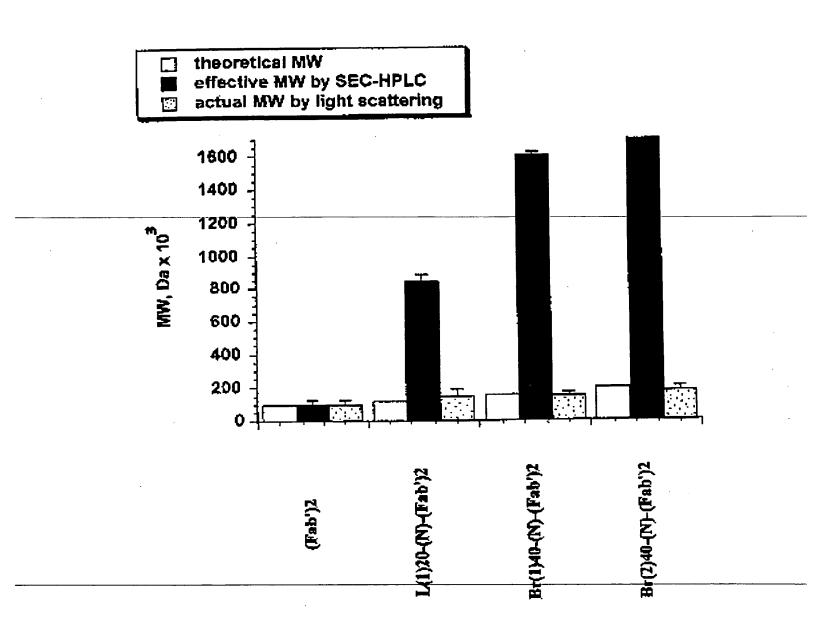


FIG. 63

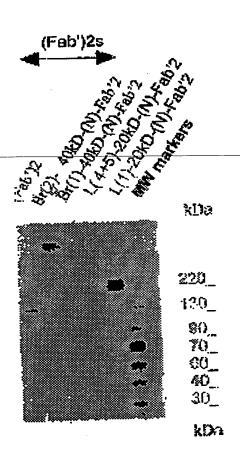
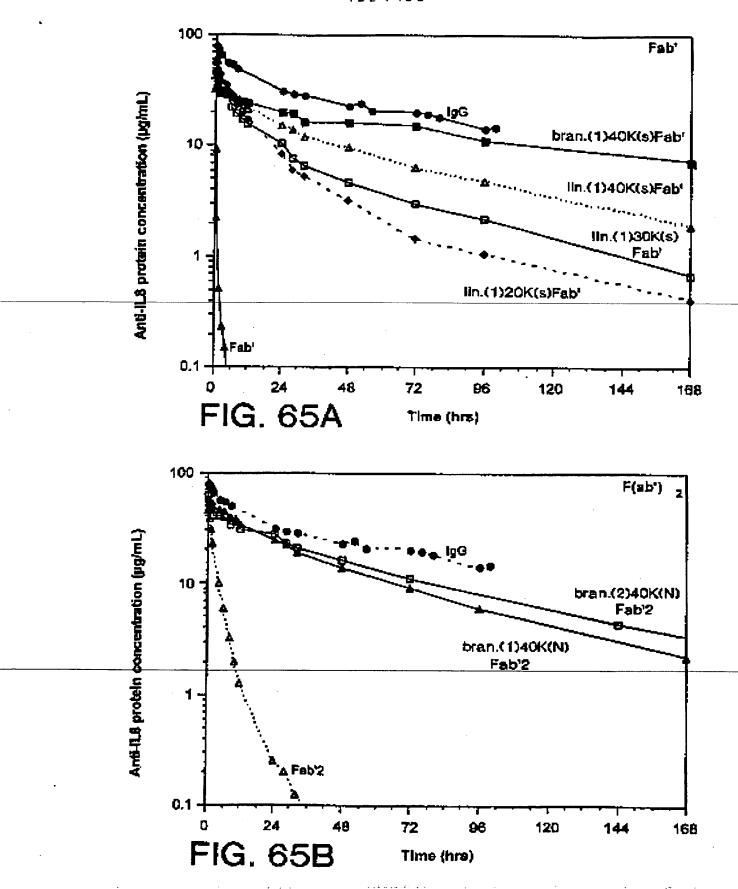


FIG. 64



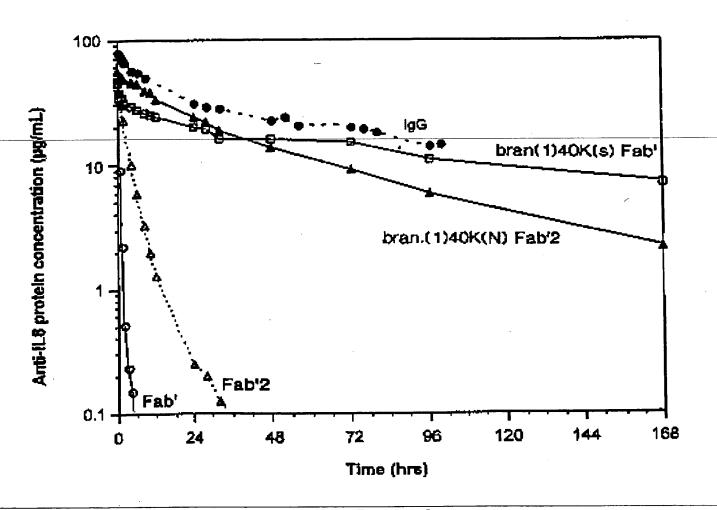
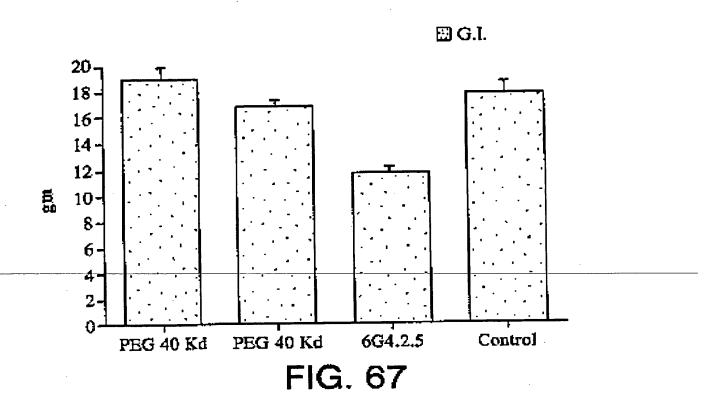
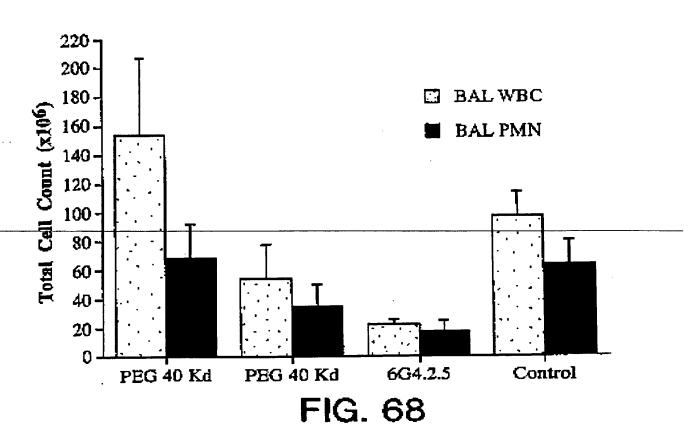


FIG. 66





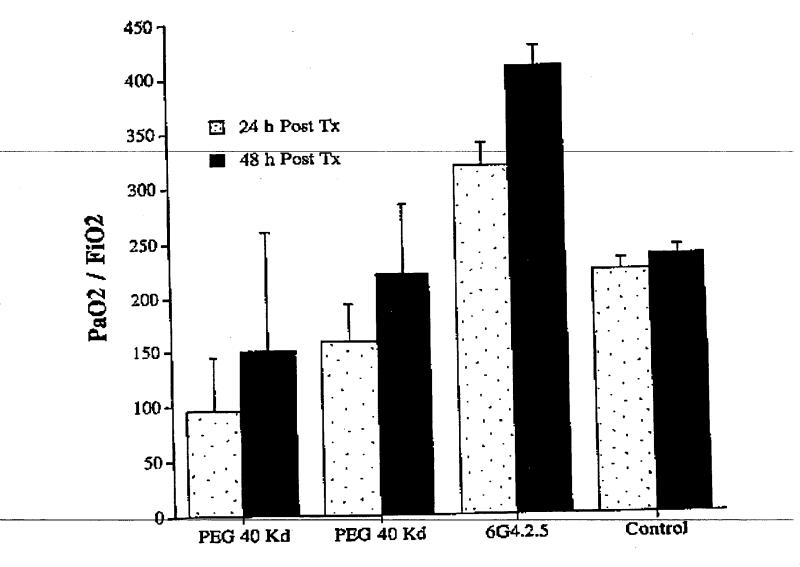


FIG. 69

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#### INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶:
C12N 15/13, C07K 19/00, A61K 47/48,
C07K 16/24, C12N 15/85, 5/10

A3

(11) International Publication Number:

WO 98/37200

(43) International Publication Date:

27 August 1998 (27.08.98)

(21) International Application Number:

PCT/US98/03337

(22) International Filing Date:

20 February 1998 (20.02.98)

(30) Priority Data:

08/804,444 09/012,116 21 February 1997 (21.02.97)

22 January 1998 (22.01.98)

US US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application

US

09/012,116 (CIP)

Filed on

22 January 1998 (22.01.98)

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(72) Inventors; and

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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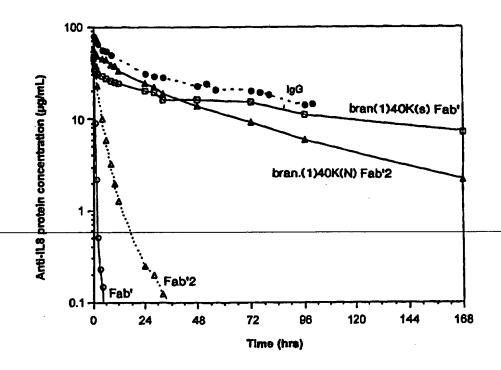
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:

28 January 1999 (28.01.99)

(54) Title: ANTIBODY FRAGMENT-POLYMER CONJUGATES AND HUMANIZED ANTI-IL-8 MONOCLONAL ANTIBODIES



#### (57) Abstract

Humanized anti-IL-8 monoclonal antibodies and variants thereof are described for use in diagnostic applications and in the treatment of inflammatory disorders. Also described is a conjugate formed by an antibody fragment covalently attached to a non-proteinaceous polymer, wherein the apparent size of the conjugate is at least about 500 kD. The conjugate exhibits substantially improved half-life, mean residence time, and/or clearance rate in circulation as compared to the underivatized parental antibody fragment.

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Intc. ional Application No PCT/US 98/03337

A. CLASS IPC 6	SIFICATION OF SUBJECT MATTER C12N15/13 C07K19/00 A61 C12N5/10	K47/48 C07	7K16/24	C12N15/85 -
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C. DOCUM	ENTS CONSIDERED TO BE RELEVANT			
Category °	Citation of document, with indication, where appropriate, o	of the relevant passages		Relevant to claim No.
A	WO 95 23865 A (GENENTECH, INC UNIVERSITY FOUNDATION) 8 Sep- see examples see claims			26-28, 35-52
A	N. KATRE: "The conjugation of with polyethylene glycol and polymers. Altering properties to enhance their therapeutic ADVANCED DRUG DELIVERY REVIEW vol. 10, no. 1, 1993, pages 9XP002084717 Amsterdam, The Netherlands see figure 3	other s of proteins potential." WS,	;	1-25,29
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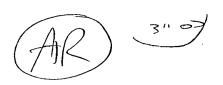
Category	Citation or document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	E. ENOAMOOOUAYE ET AL.: "Altered biodistribution of an antibody-enzyme conjugate modified with polyethylene-glycol."	1-25,29
	BRITISH JOURNAL OF CANCER, vol. 73, no. 11, June 1996, pages 1323-1327, XP002084718 London, GB see page 1324, left-hand column, line 33 - line 54	
A	E. MAINOLFI ET AL. 'REDUCTION OF IMMUNOGENICITY OF A MURINE ANTI-ICAM-1 ANTIBODY THROUGH PEGYLATION CHEMISTRY.': "In: THE 9TH INTERNATIONAL CONGRESS OF IMMUNOLOGY (abstract book), " July 1995, SAN FRANCISCO, CA, USA XP002084720	1-25,29
A	see page 885, abstract 5247  C. CUNNINGHAM-RUNDLES ET AL.: "Biological activities of polyethylene-glycol immunoglobulin conjugates."  JOURNAL OF IMMUNOLOGICAL METHODS, vol. 152, no. 2, 10 August 1992, pages 177-190, XP000471626  Amsterdam, The Netherlands see 'Material and Methods'	1-25,29
Α	C. DELGADO ET AL.: "Enhanced tumour specificity of an anti-carcinoembryonic antigen Fab' fragment by poly(ethylene glycol) (PEG) modification." BRITISH JOURNAL OF CANCER, vol. 73, no. 2, January 1996, pages 175-182, XP002084719 London, GB see the whole document	1-25,29

## INTERNATIONAL SEARCH REPORT

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Information on patent family members				PCT/US 98/03337			
Patent document cited in search report		Publication Patent to date membe		tent family ember(s)		Publication date	
WO 9523865	A	08-09-1995	CA EP JP US US US	218178 074948 950983 570762 570294 568607 567742	8 A 7 T 2 A 6 A 0 A	08-09-1995 27-12-1996 07-10-1997 13-01-1998 30-12-1997 11-11-1997 14-10-1997	
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